ASSESSMENT OF MUSCLE ACTIVATION CAPACITY: METHODOLOGICAL CONSIDERATIONS OF MUSCLE MECHANICS AND IMPLICATIONS FOR TESTING

T M BAMPOURAS

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ASSESSMENT OF MUSCLE ACTIVATION CAPACITY: METHODOLOGICAL CONSIDERATIONS OF MUSCLE MECHANICS AND IMPLICATIONS FOR TESTING

THEODOROS M. BAMPOURAS

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ABSTRACT

Muscle activation capacity assessment could be affected by the mechanical behaviour of the muscle, but this aspect has received little attention. Understanding the effect the muscle's mechanical behaviour has on muscle activation capacity assessment can be paramount in achieving a better understanding of muscle function. The aim of the present Thesis was to examine the methodological implications of the mechanical behaviour of the muscle during muscle activation capacity assessment. Four studies were designed to examine the effect of the muscle-tendon unit on a) muscle activation capacity calculation methods and number of stimuli used, by manipulating quadriceps muscle length and consequently stiffness, b) stimulation intensity required and associated discomfort, by examining whether a lower than supramaximal stimulation intensity threshold, sufficient to stretch the muscle-tendon, exists, and c) the interplay between muscle mechanics and activation, by manipulating the testing position on the dynamometer, stabilisation and concurrent activation of remote muscles. Isometric knee extensions were used for all studies, and electrical stimuli was delivered to the muscle to quantify muscle activation capacity or induce muscular contractions by circumventing the voluntary neural drive. The results showed that a) altered muscle stiffness affects muscle activation values depending on the calculation method and number of stimuli used, suggesting caution to testing where muscle stiffness is likely to change, b) a lower stimulation intensity exists that can reduce subject discomfort while obtaining valid activation capacity results, widening the application of electrical muscle stimulation, and c) muscle activation must be considered in musculoskeletal models for more accurate predictions but the level of activation will ultimately depend on how stabilised the muscle is. Collectively, these results demonstrate the considerable effect muscle mechanics have on muscle activation capacity and that muscle strength assessment must take into account this aspect for more accurate inferences on muscle function.

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Electrical muscle stimulation is a procedure that is, unfortunately, not pain free. For me to have been able to test what I wanted, decide on the correct procedures and pilot some ideas, several subjects were very patient and helpful in lending me their time, their legs and their effort. A very big ‘thank you’ to all of them for enduring all the testing and providing some very useful feedback.

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This Thesis is dedicated to my son Alexandros, in the hope that I always make him proud.

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CHAPTER 1

INTRODUCTION

The ability of skeletal muscle to generate force is a key aspect in performing activities of daily living, such as walking, going up a set of stairs, or performing sporting activities, such as jumping and kicking. Quantifying this ability, therefore, is crucial in assessing strength deficits (e.g. effect of quadriceps asymmetry on functional performance; Schmitt et al, 2012), monitoring training (e.g. power v strength squats; Brandon et al 2015) or rehabilitation (e.g. of hamstring-tendon graft anterior cruciate ligament reconstruction patients; Harput et al, 2015) programmes, or comparing populations to better understand the effects of ageing (Reeves et al, 2004) or clinical conditions (e.g. spinal cord injuries, Maganaris et al, 2006; fibromyalgia, Bachasson et al, 2013), for example.

Exerting maximal isometric force depends on a number of factors including, muscle size (Enoka, 1988), joint angle (de Ruiter et al, 2004; Pincivero et al., 2004), antagonistic co-contraction (Kubo et al, 2004) and the level of activation of the muscle (Dowling et al, 1994; Yue et al, 2000). Activation of the muscle refers to the recruitment of its motor units through increased neural drive (Taylor, 2009), and as such, is a crucial factor in force production, as diminished or increased ability to activate the muscle will result in reduced or increased force, respectively. In a study by Babault et al (2006) where the quadriceps torque and activation was measured before and after a fatiguing task (three continuous isometric contractions), they reported a ~36% decrease in activation with a corresponding ~58% reduction in torque. On the other hand, in two training studies with older individuals (Morse et al, 2005; Reeves et al, 2004), a ~10% increase in activation following the training programme was accompanied by a ~21% plantarflexors torque increase (Morse et al, 2005), while a 5% increase in muscle activation was accompanied by an ~8% isometric quadriceps torque increase (Reeves et al, 2004). The collective assumption from these studies is that strength changes are closely associated and can be largely attributable to activation changes.

Muscle ctivation capacity has also been examined for different populations. For example, activation has been suggested as a key factor in evaluating muscle quality and function in ageing (Klass et al, 2007; McGregor et al, 2014), as lower activation would point towards a diminished ability of neural control. Morse et al (2004) examined older individuals (average age 73.7 years) and reported that the 39% reduced isometric plantarflexion torque in older compared to their younger (average age 24.7 years) counterparts was not only due to loss of muscle mass but also due to a 19% reduced ability to activate the muscle. In another study, older individuals (average age 68 years) and middle-aged individuals (average age 48 years) had 13% and 8% lower plantarflexion activation capacity, respectively, compared to young individuals (average age 24 years) during an isometric plantarflexion task (Onambele et al, 2006). Finally, Stevens et al (2001), reported higher activation values of 98.1% for a young group (average age 23.7 years) compared to 95.5% for the older group (average age 73.2 years) during an isometric knee extension.

Other examples of the use of muscle activation in comparing the ability to exert maximal force include comparisons between individuals with low and high body fat percentage (Tomlinson et al, 2014), as well as fibromyalgia patients and healthy controls (Bachasson et al, 2012). Comparisons between young individuals with body fat % > 40 to those with body fat % < 40, showed that those with the higher body fat percentage were less able to activate their plantarflexor muscles by 6.4%, suggesting muscle weakness in obese individuals is, to an extent, the result of reduced neural drive (Tomlinson et al, 2014). In contrast, fibromyalgia patients had similar activation levels but lower isometric knee extension torque, suggesting muscle mass and not neural reasons for the reduced strength generation ability (Bachasson et al, 2012). In another patient population, rheumatoid arthritis patients demonstrated similar activation levels and force to age- and sex-matched healthy controls during an isometric knee extension, leading to the conclusion that their muscle properties are maintained and the patients can train in a similar way to healthy individuals (Matschke et al, 2010). Such results help better understand any weakness associated with the effect e.g. ageing or clinical conditions have on neural drive to the muscle, separate the neural changes from the morphological and physiological changes, and design appropriate interventions to address any weakness (Matschke et al, 2010; Stevens et al, 2001).

Quantifying, therefore, the level of activation is fundamental in the measurement of muscle force (Folland and Williams, 2007) since this quantification can suggest how much muscle weakness can be attributed to the inability of the central nervous system to fully recruit motor units (Lewek et al, 2004), enabling correct inferences about muscle function. This quantification of activation level is typically performed during and via the measurement of isometric torque produced by an isometric maximum voluntary contraction (MVC) on a dynamometer. As already seen in several abovementioned studies, the quadriceps muscle group is frequently assessed, due to its important role in fundamental activities for all ages (Hurley et al, 1998; Knight and Kamen, 2001). Indeed, such assessment has been applied to various clinical (e.g. Hart et al, 2010), older age (e.g. Reeves et al, 2004) or athletic (e.g. Maffiuletti et al, 2000) populations.

Quantification of muscle activation is possible when an electrical impulse is applied to the muscle during an MVC (Merton, 1954). The application of electrical stimulation attempts to recruit the fibres that were left inactive by the voluntary contraction (Behm et al, 1996; de Ruiter et al, 2004; Kooistra et al, 2007), providing an indication of the true maximal force (Allen et al, 1995; Miller et al, 2006). If the muscle is not fully activated, a twitch-like force increment is produced, indicating recruitment of the previously inactive muscle fibres (Belanger and Comas, 1981). This increase in force beyond the maximum voluntary force becomes smaller or completely diminishes with increasing neural drive to the muscle, indicating full activation through volition (Shield and Zhou, 2004) (Figure 1). This notion has been widely applied to assess activation capacity of different populations, such as clinical patients (Rutherford et al. 1986; Suter et al, 1998), children (O'Brien et al, 2008), athletes (Huber et al, 1998; Maffiuletti et al, 2000) or the elderly (Kent-Braun and Ng, 1999; Reeves et al, 2003; Morse et al, 2004).

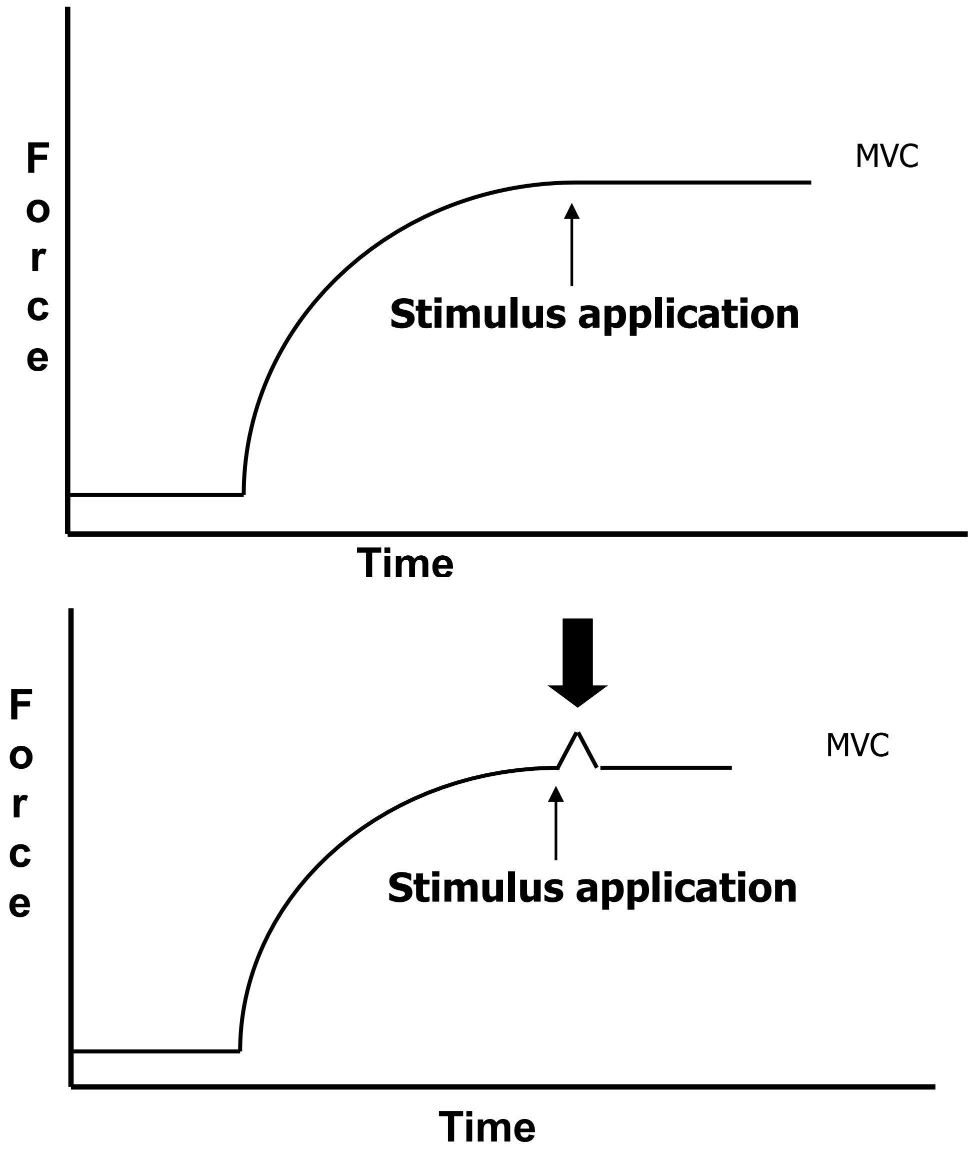

Figure 1. A schematic diagram of the muscle activation capacity assessment concept. When a stimulus is applied during a maximum voluntary contraction (MVC) and no increase in force occurs (top panel), then the muscle is assumed to be fully activated. However, when the applied stimulus results in an increase in force (bottom panel), then the muscle is not fully activated and the magnitude of the increase can be used to quantify the activation level.

Based on this principle, Merton (1954) proposed a method to quantify muscle activation, the interpolated twitch technique (ITT) (for a review, please see Shield and Zhou, 2004). This method involves the delivery of an electrical stimulus (or series of stimuli) to the muscle or parent nerve during an MVC (superimposed stimulus) and the application of an identical electrical stimulus at rest (resting stimulus). Once the superimposed and resting stimuli are measured, muscle activation can then be calculated as

(1 – (superimposed stimulus magnitude / resting stimulus magnitude)) x 100

In contrast, other researchers (e.g. Kent-Braun and Ng, 1999; Reeves et al, 2003; Roberts et al, 2012; Otzel et al, 2015; Pamukoff et al, 2016) have used the central activation ratio (CAR) method, which takes into account only the MVC and the superimposed stimulus magnitude and is calculated as

(MVC magnitude / MVC and superimposed magnitude) x 100.

Notwithstanding the importance of assessing activation levels as part of the muscle's capabilities, methodological concerns have been raised with regards to the assessment and the use of electrical muscle stimulation (de Haan et al, 2009). Although the validity (de Haan et al, 2009) and reliability (Allen et al, 1995; Morton et al, 2005) of the method have been verified, the sensitivity of the measurement has been challenged (Herbert and Gandevia, 1999). This can be better illustrated when one considers the findings from two studies (Knight and Kamen, 2001; Stevens et al 2001), both comparing activation capacity of young and older individuals of similar ages for both groups during an isometric knee extension contraction. Stevens et al (2001) reported statistically significantly higher activation values of 98.1% for the young group (average age 23.7 years) compared to 95.5% for the older group (average age 73.2 years); a difference of 2.6%. In contrast, Knight and Kamen (2001) reported similar activation values between young (average age 21.4 years, 95% activation) and older (average age 77 years, 97% activation) individuals; a difference of 2%.

Another concern with the ITT method is the assumption of linearity in the relationship between the superimposed and resting twitches. However, several studies have demonstrated that this relationship is non-linear (e.g. Folland and Williams, 2007; Scaglioni and Martin, 2009), with aspects such as lower contraction intensities (Behm et al, 1996; Yue et al, 2000), antidromic potentials (Herbert and Gandevia, 1999), antagonistic co-contraction (Folland and Williams, 2007) or compliance of experimental and biological structures (Allen et al, 1995; Loring and Hershenson, 1992) contributing towards that relationship. This relationship makes it hard to predict the real maximum force, as both underestimation (De Serres and Enoka, 1998) and overestimation (Herbert and Gandevia, 1999) have been reported. Nonetheless, this aspect only brings into question the extrapolation validity rather than the validity of the technique itself (Shield and Zhou, 2004), and it can thus be considered as a valid measure of voluntary drive to the muscle (Taylor, 2009).

The measurement sensitivity of muscle activation capacity assessment using electrical muscle stimulation has been suggested to be affected by both the resting and superimposed twitches' ability to respond consistently to stimulus (Folland and Williams, 2007), thus impacting on both ITT and CAR calculation methods. Application of higher number of stimuli / tetanus superimposed on the MVC, should result in higher muscle recruitment and, thus, a superimposed twitch of increased torque magnitude. Consequently, this would result in a reduced muscle activation value with the CAR method. Similarly, the use of a resting twitch applied prior to the MVC compared to a resting twitch applied after the MVC could potentially affect the activation capacity result obtained with ITT, as the pre-MVC twitch would be unpotentiated while the post-MVC one would be potentiated and therefore of higher magnitude (Kufel et al, 2002; Folland and Williams, 2007). These two studies (Kufel et al, 2002; Folland and Williams, 2007) confirmed the higher magnitude of the potentiated twitch as well as that it was less varied in results. In attempting to find appropriate stimulation parameters to ensure the correct response by the superimposed twitch is achieved, several other studies have examined activation levels with the stimulus delivered to the nerve or the muscle (Rutherford et al, 1986; Behm et al, 1996; Saglioni and Martin, 2009), different stimulation intensities (Bülow et al, 1993; Binder-Macleod et al, 1995; Valli et al, 2002; Doucet and Griffin, 2008), or number of twitches (Kent-Braun and Le Blanc, 1996; Miller et al, 1999; Suter and Herzog, 2001; Binder-Macleod et al, 2002).

Notwithstanding the neurophysiological approaches of the above studies, one aspect that is generally accepted that it can impact on the results of the assessment is the mechanical behaviour of the quadriceps during activation assessment testing (Enoka, 1988; Becker and Awiswus, 2001; Babault et al, 2003; de Ruiter et al, 2004; Shield and Zhou, 2004; de Haan et al, 2009; Taylor, 2009). For example, the length of the quadriceps muscle-tendon unit has been shown to affect activation levels (Becker and Awiswus, 2001; Babault et al, 2003; Newman et al, 2003; de Ruiter et al, 2004), however, the direction of that effect is unclear, with activation capacity reported as higher with longer muscle lengths (Becker and Awiswus, 2001; de Ruiter et al, 2004), shorter muscle lengths (Babault et al, 2003) or no differences in activation between different muscle lengths (Newman et al, 2003). Given that for a complete and accurate force assessment, the mechanical behaviour of the quadriceps muscles is paramount, further, more holistic examination of the mechanical implications on muscle activation capacity is warranted. The potential avenues in which muscle mechanics can impact on muscle activation capacity assessment are presented below.

Muscle activation capacity assessment calculation

Both ITT and CAR muscle activation capacity assessment methods are based on the principle that the muscle is not fully activated, if the superimposed stimulus causes an increase in force. However, the quantitative agreement between these two methods has received little attention (Klass et al, 2007). An initial comparison of the two methods was carried out by Behm et al (2001), and reported higher muscle activation values for CAR compared to ITT for the same knee joint angle. The discrepancy between the two methods was attributed to the consideration of the resting stimulus in the respective equations (as the resting stimulus is considered in the ITT method but not in CAR).

Loring and Hershenson (1992) reported that a reduced series compliance of a testing apparatus when assessing the adductor pollicis resulted in higher twitch magnitude, suggesting that a more compliant muscle would yield erroneous activation values. This notion was supported further by Becker and Awiszus (2001) and Kubo et al (2004), who examined muscle activation capacity at shorter and longer musle lengths (by manipulating the knee joint angle). Both studies concluded that activation in longer muscle lengths (i.e. stiffer muscle) was higher than in lower muscle lengths (i.e. more compliant muscle). Altering the compliance of the muscle-tendon unit, will affect transmission of force generated by the contraction (Herbert et al, 2002). Although CAR should be largely unaffected by this factor, ITT would be affected because the altered compliance of the muscle-tendon unit will impact on the resting stimulus' torque, considered in the ITT calculation only.

The number of stimuli used can yield different activation values, leading to incorrect assessment results and difficulty in comparing findings. Kent-Braun and Le Blanc (1996) examined muscle activation of healthy subjects, patients with amyotrophic lateral sclerosis and healthy subjects after fatiguing exercises using single, double or a train of stimuli. They reported that the train of stimuli produced higher force increases (i.e. lower activation values) for all conditions. The number of stimuli should not affect activation results when using the ITT method, as the increase in force of the superimposed twitch produced by the increased number of twitches superimposed on the MVC would also be evident on the resting twitch. Indeed, Allen et al (1998) reported no change in muscle activation of the elbow flexor muscles in a single, paired or trains of four stimuli when the ITT method was used. These findings agree with Behm et al (1996) who also reported no difference in muscle activation values of the plantar flexors and knee extensors with single, double or quintuplets applied and the ITT method was used.

Although the abovementioned studies have individually examined the effects of stimuli number or the agreement between the ITT and CAR methods, a conclusive answer to these issues that would make a direct comparison between the two methods, at different joint angles (thus manipulating muscle length and, in turn, muscle stiffness) and different stimuli numbers (thus ensuring ability to detect the superimposed twitch), would be of importance to both clinical and exercise studies where interventions, likely to alter the mechanical properties of the muscle-tendon unit (e.g. Zghal et al, 2014; Reeves et al, 2003), are used. The present work is addressing this need by completing this more holistic examination, providing recommendations for future studies.

Volitional effort during muscle activation capacity assessment

Quadriceps muscle activation can be applied either through stimulation of the femoral nerve or through direct stimulation of the muscle by attaching electrodes on the skin above the muscle. Nerve stimulation is possibly the only time that true maximal activation is achieved (Bigland-Ritchie et al, 1978), as the whole muscle can be activated. Percutaneous stimulation, although spreading of the stimulating current to other muscles is possible (Becker and Awiszus, 2001), it may only activate the portion of the muscle under the area of the electrodes. However, the discomfort of nerve stimulation is high and not well-tolerated by subjects (Rutherford et al, 1986), while locating and stimulating the nerve is at times difficult.

As a result of the abovementioned issues with nerve stimulation, percutaneous stimulation has been more widely used. Given the potential for not fully activating the muscle with this stimulation method, studies assessing muscle activation capacity, have typically used supramaximal stimulation intensity (Allen et al, 1995; Kent-Braun and Le Blanc, 1996; De Serres and Enoka, 1998; Behm et al, 2001; Babault et al, 2003). Supramaximal stimulation intensity is defined as the point where no further increase in muscle force is generated despite an increase in stimulation intensity applied to the muscle (Reeves et al, 2003; Morse, 2004; O'Brien et al, 2008). Nonetheless, supramaximal intensity has also been reported to induce high discomfort to subjects (Chae et al, 1998; Valli et al, 2003; Morton et al, 2005; Button and Behm, 2008;).

The discomfort induced by electrical stimulation can lead to questionable outcomes in muscle activation capacity values, due to the apprehension of subjects to exert maximal force when anticipating a painful stimulus (Button and Behm, 2008). If participants are apprehensive about exerting maximal effort during their contraction, then they are likely to not activate their muscles fully (Button and Behm, 2008). This will affect activation calculations producing inaccurate results (Luc et al, 2016; Button and Behm, 2008). Indeed, high levels of discomfort during muscle stimulation were reported (Chae and Hart, 1998; Valli et al, 2002) and suggestions were made for protocols that can reduce discomfort while validly assessing muscle activation (Miller et al, 2006; Scaglioni and Martin, 2009).

The stimulus intensity is a key factor in inducing discomfort. Although reducing the intensity is likely to reduce the discomfort, reduced intensity is also likely to produce different activation results, thus affecting the assessment. Rutherford et al (1986), however, examined percutaneous and nerve stimulation and concluded there was no difference in muscle activation capacity between the two methods. These results were supported by Scaglioni and Martin (2009), who also reported comparable results in plantar flexors activation capacity between nerve and muscle stimulation. These findings indicate that as long as the same portion of muscle is activated when the stimulus is applied during contraction and during rest, muscle activation assessment should not be affected when the ITT method is used. This would not be the case for CAR, however, as differences in stimulation method are likely to result in different superimposed twitch magnitudes and, without the presence of a normalising resting twitch, in different activation capacity results, highlighting the importance of sufficiently stretching the resting twitch. It is likely, therefore, that there could be an intensity threshold, lower than supramaximal stimulation intensity (Nashed et al, 2009), that could stretch the muscle-tendon unit sufficiently, resulting in comparable scores to supramaximal stimulation intensity and reliable activation values. Further examination of this aspect of muscle activation, along with the potential reduction in subject discomfort, can provide useful information both for clinical and research settings but also for addressing ethical and moral concerns.

Interplay of activation level and force generation

It has been previously reported that when quadriceps are at longer lengths, higher activation capacity was demonstrated (Suter and Herzog, 1997; Becker and Awiszus, 2001; Kubo et al, 2004). Some of the reasons provided to explain this difference in activation as a function of the knee joint angle were increased intra-articular pressure and ligament strain in extended knee positions (Suter and Herzog, 1997), longer muscle spindle lengths resulting in increased Ia input and, consequently, in increased excitatory drive (Becker and Awiszus, 2001), or muscle-tendon compliance at shorter muscle lengths affecting the resting twitch and, consequently, the activation capacity calculation (Loring and Hershenson, 1992).

These studies examined the different quadriceps length by manipulating the knee joint angle alone. However, the length of the rectus femoris, a bi-articular muscle and responsible for ~17% of the quadriceps torque (McNair et al, 1991), can also be manipulated through changes in hip joint angle. Indeed, studies have used different hip joint angles (e.g. 90° Dewhurst et al, 2010; 100° de Ruiter et al, 2004; 110° Kooistra et al, 2007 - full hip joint extension = 180°), inducing different activation capacity as an aspect that could affect the assessment, in addition to the quadriceps muscle length change.

Interestingly, manipulation of the hip joint angle obtains quadriceps muscle force results that present a discrepancy between musculoskeletal models and experimental studies. Musculoskeletal models predict that rectus femoris, and consequently the quadriceps muscle, will produce higher torque at longer lengths (Herzog and te Keurs, 1998; Herzog et al, 1990). In other words, as the vastii muscles are uniarticular and not affected by the hip joint angle, the supine position (lengthened rectus femoris) should generate higher torque. However, this is not verified by experimental studies, which have shown that the seated position generated higher quadriceps torque compared to supine (Maffiuletti and Leppers, 2003; Rochette et al, 2003) with reduced EMG activity at that position.

This discrepancy, therefore, can be attributed to two possible reasons, reduced muscle activation capacity at the longer rectus femoris length but also increased antagonistic co-activation. Reduced muscle activation capacity, as indicated earlier on, has been previously shown to occur at shorter rather than longer muscle lengths. With the hip joint angle change, there is also alteration in the length of the biarticular hamstrings muscle. By lengthening (seated) or shortening (supine) the hamstrings muscles, the different muscle length as well as different activation capacity of the antagonist muscle, could impact on the 'net' torque achieved at the isometric knee extension.

Reduced activation capacity could be the result of lack of sufficient stabilisation of the pelvis. Lack of stabilisation during the contraction can impact on the assessment of muscle strength through different ways. On one hand, lack of stabilisation of the pelvis during the assessment, can create the need for the biceps femoris muscle to contract more to stabilise the pelvis (van Wingerden et al, 2004), thus potentially reducing the agonist activation during reciprocal inhibition (Hamm and Alexander, 2010). On the other hand, in order to increase stabilisation, subjects typically contract other muscles, remote to the muscle of interest. This fixation to the dynamometer chair appears to enable increased torque production (Hart et al, 1984; Magnusson et al, 1993). However, this increased torque could be due to the increased activation of the tested muscle through the activation of these remote voluntary contractions; concurrent activation potentiation (CAP; Ebben et al, 2008). It is unclear whether these remote voluntary contractions can increase quadriceps torque through stabilisation or activation capacity.

Clarification of these issues will provide us with a more standardised approach to muscle function assessment, but also with an insight for more realistic musculoskeletal model development.

CHAPTER 2

THESIS STRUCTURE AND PRESENTATION

To address the problems developed earlier and examine the effect of muscle mechanics on muscle activation capacity, four studies were carried out, and a summary for each is presented below. These studies are presented in the Thesis as experimental standalone papers in the next four chapters.

Study 1

This study aimed to compare the effect of the muscle activation capacity calculation method used, number of stimuli and knee joint angle on muscle activation assessment. Muscle activation was assessed with a singlet, doublet, quadruplet or octuplet electrical twitch, delivered during the plateau phase of an isometric MVC and at rest. In addition, the stimulation took place at two different knee joint angles, 30° and 90°. The order of number of stimuli as well as knee joint angle were randomised.

Measurements of maximal contractile torque and voluntary activation were made. Isometric MVC torque as well as the superimposed twitch torque were measured with the use of an isokinetic dynamometer. Muscle activation was calculated with both methods (ITT and CAR) for all stimuli number and joint angles. A mixed design 2 (muscle activation assessment method) x 2 (knee joint angle) x 4 (number of stimuli) was used to examine for any differences.

Study 2

This study aimed to determine whether submaximal stimulation intensity can yield similar results to supramaximal intensity and if so, at what percentage of the supramaximal twitch this can occur. Muscle activation was assessed with electrical twitches during an isometric MVC and at rest, with the knee joint angle at 90^{o}. The force produced by supramaximal stimulation intensity (force produced considered 100%) was recorded and 10%-90% levels (in increments of 10%) of that were calculated. Subsequently, the submaximal stimulation intensity that produced each level of force, was identified and recorded. Muscle activation capacity was then assessed at all submaximal stimulation intensities, in a randomised order.

Measurements of maximal contractile force and voluntary activation were made. Force of the isometric MVC as well as the superimposed twitch force were measured with the use of a custom-made isometric dynamometer. In addition, assessment of discomfort at each percentage was also assessed with the use of a visual analog scale (VAS). Muscle activation was calculated with ITT. The full curve of muscle activation values was plotted for the different stimulation intensities (10%-100% of stimulation intensity) and Dunnett's test (comparing each stimulation intensity activation or discomfort score to the supramaximal one) was used to identify the lowest stimulation intensity that produces comparable results to the supramaximal stimulation intensity.

Study 3

This study aimed to examine whether the discrepancies in musculoskeletal modelling studies and experimental studies can be attributed to differences in muscle activation or antagonistic co-contraction. The knee joint angle was maintained the same at 90° but the hip joint was manipulated into two different positions, seated and supine (160°, full hip joint extension = 180°). Subjects performed isometric quadriceps MVCs in both positions. Additionally, tetanic stimulation took place during rest in both positions, to allow comparisons of the muscle's force generating ability based on its mechanical aspect only.

Maximal contractile quadriceps torque was recorded, with the use of an isokinetic dynamometer, while antagonistic co-activation torque during the isometric quadriceps MVC was estimated with the use of EMG. Subsequently, 'corrected' MVC (isometric quadriceps MVC plus antagonistic torque) was calculated. Paired comparisons between the two positions were made for the quadriceps MVC torque, corrected torque, antagonistic co-activation torque and tetanic stimulation torque, to examine for differences between the two positions.

Study 4

This study aimed to examine further the effects of stabilisation on the dynamometer seat during isometric quadriceps contractions. The subjects' testing position was seated throughout the experiment, however the stabilisation applied as well as the muscles activated during the experiment differed. In addition to a typical MVC, subjects performed another MVC while also contracting the forearm muscles by squeezing maximally a handgrip dynamometer, an MVC while they were not restrained and an MVC where they were restrained, by being asked to contract their leg muscles only. EMG from eleven muscles was used to assess muscle activity but also confirm that instructions were followed.

Maximal contractile quadriceps torque and EMG was recorded, with the use of an isokinetic dynamometer, while muscle activation capacity was calculated. A 1 x 4 (condition) repeated measures ANOVA, with pairwise comparisons for differences and corrected for multiple comparison were conducted for MVC and activation. Friedman's test, with Wilcoxon test for differences, also adjusted using Holm-Bonferroni, were used to examine for EMG differences between conditions.

CHAPTER 3

MUSCLE ACTIVATION ASSESSMENT: EFFECTS OF METHOD, STIMULI NUMBER AND JOINT ANGLE

A version of the work from this chapter has been published as:

Bampouras TM, Reeves ND, Baltzopoulos V, Maganaris CN. Muscle activation assessment: effects of method, stimuli number and joint angle. Muscle Nerve 2006; 34(6):740–746. (Appendix 1)

ABSTRACT

The aim of this study was to compare and assess the measurement sensitivity of the interpolated twitch technique (ITT) and central activation ratio (CAR) to potential errors introduced by 1) evoking inadequate force, by manipulating the number of stimuli and 2) neglecting differences in series elasticity between conditions, by manipulating joint angle. Ten subjects performed knee extension contractions at 30 and 90 degrees knee joint angles during which the ITT [(1-superimposed stimulus torque/resting stimulus torque) x 100] and CAR [voluntary torque/voluntary torque+superimposed stimulus torque] methods were applied using 1, 2, 4 and 8 electrical stimuli. Joint angle influenced the ITT outcome with higher values taken at 90 degrees ($p < 0.05$), while stimuli number influenced the CAR outcome with a higher number of stimuli yielding lower values ($p < 0.05$). For any given joint angle and stimuli number, the CAR method produced higher activation values than the ITT method by 8-16%. Therefore, it is suggested that in the quantification of voluntary drive with the ITT and CAR methods consideration be given not only to the number of stimuli applied but also to the effect of series elasticity due to joint angle differences, since these factors may affect differently the outcome of the calculation, depending on the approach followed.

INTRODUCTION

The measurement of isometric torque produced by maximal effort voluntary contraction (MVC) has been routinely used for assessing muscle function in different populations, such as patients with fibromyalgia and anterior knee pain (Noregaard et al, 1994; Suter et al, 1998), elderly (Kent-Braun and Ng, 1999; Reeves et al, 2003) and athletes (Huber et al, 1998; Maffiuletti et al, 2000), and after b) acute (e.g., fatigue (Kawakami et al, 2000; Newman et al, 2003)) and chronic interventions (e.g., exercise training (Colson et al, 2000; Maffiuletti et al, 2002)). One of the main factors that affect MVC torque generation is the degree to which the agonist muscles tested are activated by volition. This functional parameter shows physiological variation in clinical situations such as motor neuron disorders (Kent-Braun and Le Blanc, 1996; Rutherford et al, 1986) and joint pathologies (Norregaard et al, 1994; Suter et al, 1998). However, it may also be subject to methodological variation (Allen et al, 1998; Behm et al, 2001; Oskouei et al, 2003).

To assess activation capacity, two methods have traditionally been employed: The interpolated twitch technique (ITT) (Allen et al, 1995; Becker and Awiszus, 2001; Behm et al, 2001; Behm et al, 1996; Dowling et al, 1994; Huber et al, 1998; Maffiuletti et al, 2002; Oskouei et al, 2003; Shield and Zhou, 2004; Suter and Herzog, 2001) and the central activation ratio (CAR) (Kent-Braun and Ng, 1999; Merton 1954; Reeves et al, 2003). The ITT method involves the application to the muscle or parent nerve of an electrical stimulus (or series of stimuli at frequencies allowing a fused contractile response) during an MVC (superimposed stimulation) and the application of an identical electrical stimulus at rest. Activation capacity with this method is calculated as

ITT = (1- (superimposed stimulus torque / resting stimulus torque)) x 100 [equation 1].

The CAR method involves only the application of a superimposed stimulus and the activation capacity is calculated as

CAR = MVC torque / (MVC torque + superimposed stimulus torque) [equation 2].

Despite the fact that both methods are based on the principle that activation is incomplete if the superimposed stimulation causes any further torque increase to the MVC, the quantitative agreement of the two methods and the mechanisms underpinning any possible differences have not been fully elucidated. To date, only Behm et al (2001) have compared the two methods and found that the CAR method yielded higher activation values than the ITT method when applied at the same joint angle.

Surprisingly, however, superimposing two stimuli or a tetanus at 100 Hz produced similar CAR values (Behm et al, 2001). Increasing the number of stimuli would be expected to increase the extra contractile torque produced by the superimposed stimulus due to summation of twitch contractile responses, and should thus result in reduced activation capacity values using the CAR method. In contrast, this would not be the case for the ITT method, since this method encompasses also the torque produced by applying the same stimulus at rest. Errors of different magnitude might also be introduced in the two methods when comparative measurements are taken across a range of joint positions. Changes in joint angle would alter the passive stiffness of the series elastic component (SEC) of the muscle. This alteration may result in changes in the effectiveness of the SEC to transmit the force evoked by the application of an electrical stimulus to the muscle, thus potentially affecting the magnitude of the resting twitch and consequently the calculation of activation capacity using the ITT method. In contrast, the lack of resting twitch in the CAR method would render this method insensitive to errors associated with changes in SEC stiffness with joint angle.

To gain insight into the above methodological issues and their impact on the estimation of activation capacity, the present study aimed to compare the ITT and CAR methods when manipulating the number of electrical stimuli and joint angle. The quadriceps muscle group was studied and it was hypothesized that, for a given level of volitional effort during knee extension contraction, a) the CAR method would be more sensitive than the ITT method to differences in the number of applied stimuli, and b) the ITT method would be more sensitive than the CAR method to knee joint angle changes for any given number of stimuli.

METHODS

Ten healthy, physically active males (age: 29 ± 7 years, height: 178 ± 6 cm, body mass: 78 ± 10 kg; mean ± SD) provided written informed consent to participate in this study, which was approved by the Institutional Ethics Committee. All subjects were tested in the laboratory on a single occasion, but had previously visited the laboratory on at least another one occasion to become familiar with the experimental procedures involved.

Knee extension MVC torque was measured on the right leg at knee joint angles of 30 and 90 degrees (full knee extension = 0 degrees) with the hip joint at 85 deg (supine position = 0 degrees), using an isokinetic dynamometer (Cybex NORM, Ronkonkoma, NY). The knee joint angles tested were selected in order to represent positions where the passive stretch applied to the SEC varied considerably (Becker and Awiszus, 2001; Kooistra et al, 2007), i.e., the SEC is stretched at 90 deg and slacker at 30 deg. The centre of rotation of the knee was aligned with the dynamometer axis. Straps were positioned at the hip, shoulders and over the tested thigh to prevent extraneous movement. The subjects were instructed to perform all contractions by increasing their effort gradually in ~2-3 s and maintain the maximum torque produced for an additional ~1 s. A rest period of 2-3 minutes separated the contractions.

The quantification of voluntary activation during the MVCs was based on the application of electrical stimulation. Femoral nerve stimulation proved to cause major discomfort in some subjects, especially when applying trains of stimuli; hence percutaneous muscle stimulation was preferred. Two 7 x 12.5-cm self-adhesive electrodes were placed on the proximal and distal regions of the quadriceps muscle group. The size and location of the stimulating electrodes were determined in preliminary experiments, with the criterion being the generation of the highest possible knee extension torque at each angle by applying a twitch of a given intensity. Signals of torque and electrical stimuli application were displayed on the screen of a computer (Macintosh, G4, Apple Computer, Cupertino, CA, USA), interfaced with an acquisition system (Acknowledge, Biopac Systems, Santa Barbara, CA, USA) used for analog-to-digital conversion, at a sampling frequency of 2,000 Hz. Stimuli of 200-μs pulse width and 10-ms inter-stimulus gap were generated by an electrical stimulator (model DS7, Digitimer stimulator, Welwyn, Garden City, UK) modified to deliver a maximum of 1,000 mA output. One (singlet), two (doublet) and four stimuli (quadruplet) were applied in a randomized order in all ten subjects. Six of the ten subjects were capable of tolerating discomfort levels caused by application of eight stimuli (octuplet); hence, these data were also collected and included in the analysis.

The supramaximal stimulation intensity was determined at each knee joint angle by single twitches applied at rest with increasing current intensity at 300 V. Supramaximality was defined as the level at which a further increase in current of 50 mA did not elicit an increase in twitch torque. Supramaximal stimulation was applied during the plateau phase of MVC and 3 s after complete relaxation following the MVC (Figure 2). The latter resting potentiated stimulus was evoked automatically. Two MVCs were performed and the contraction with the highest torque was selected for analysis.

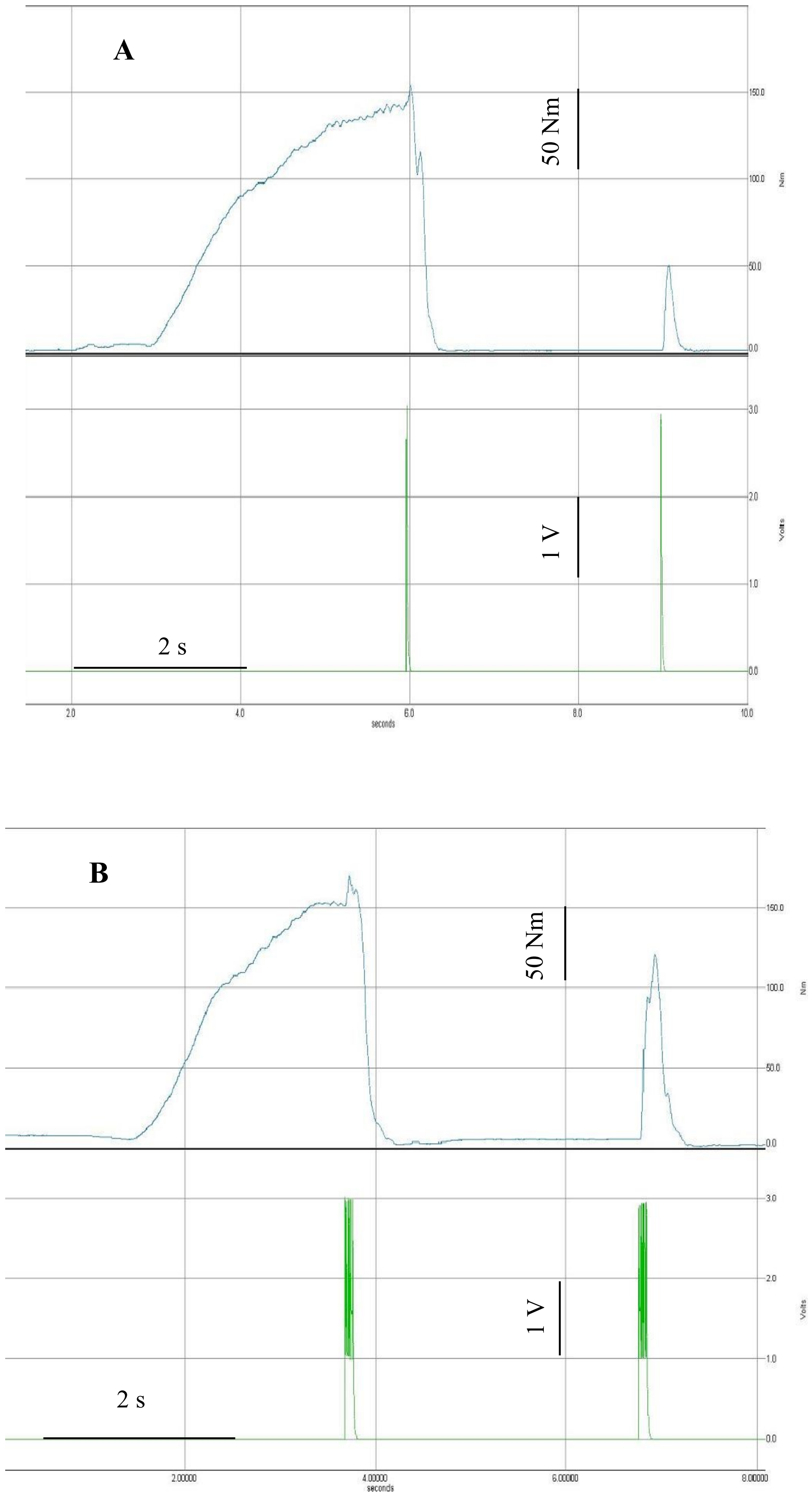

Figure 2. *Top*: Torque traces for one participant at 90 degrees knee joint angle during application of a singlet (A) and an octuplet (B). *Bottom*: Time of stimuli application in the above contractions.

Activation capacity was calculated from equation 1 for the ITT method and equation 2 for the CAR method (see Introduction). The rate of torque development (RTD) for the resting stimuli was measured to further elucidate the influence of SEC on the ITT method's outcome. Rate of torque development for each stimuli number was measured as the gradient of the torque-time curve from rest to peak torque during stimulation.

Normality of the data was examined using the Kolmogorov-Smirnov test. In cases where the data were not normally distributed, a transformation was performed using the most appropriate transformation function (Tabachnick and Fidell, 2000) prior to further analysis and normality was subsequently confirmed. A repeated measures analysis of variance (ANOVA) was used to examine for differences in baseline MVC torque at each joint angle, just before superimposing the singlet, doublet, quadruplet and octuplet. ANOVA was also used to examine for differences in the torque ratio of superimposed stimulation to resting stimulation between the singlet, doublet, quadruplet and octuplet at each joint angle. A 2 x 2 x 4 repeated measures factorial ANOVA was used to examine for differences in activation capacity between methods, knee joint angles and stimuli number. A 2 x 4 repeated measures factorial ANOVA was used to examine for differences in the rate of torque development between stimuli number and joint angle. Simple effects tests were used for post hoc analysis where appropriate. Values are presented as the mean ± SD. Significance was accepted at the level $p < 0.05$.

RESULTS

The torque values produced prior to superimposed stimulation at each joint angle were not different between contractions ($p > 0.05$; Table 1), indicating that the volitional efforts exerted during the MVCs were similar. The current corresponding to supramaximal stimulation intensity was identical for the two joint angles (731 ± 92 mA).

Table 1. Summarized data (mean ± SD) for maximum effort voluntary contraction (MVC), torque ratios of resting stimulation to superimposed stimulation (TR) and rate of torque development (RTD) for both joint angles and all stimuli number. *indicates significant difference between knee joint angles

	Joint angle (degrees)	Singlet	Doublet	Quadruplet	Octuplet
MVC (Nm)	30	129.4 ± 41.5	133.2 ± 39.9	135.3 ± 38.2	153.2 ± 49.9
	90	208.6 ± 42.7	203.3 ± 41.5	197.4 ± 37.6	181.0 ± 42.2
TR	30	7.5 ± 5.5	7.5 ± 5.2	4.3 ± 1.7	5.4 ± 3.8
	90	10.9 ± 6.0	8.5 ± 4.1	14.6 ± 16.1	19.8 ± 33.2
RTD (Nm/s)	30	273.4 ± 162.5	437.3 ± 263.6	602.5 ± 318.3	754.8 ± 424.1
	90	578.3 ± 378.5*	821.5 ± 569.9*	869.3 ± 628.3*	805.0 ± 556.1

There was no effect of stimuli number on the ITT outcome for either joint angle ($p > 0.05$). In contrast, there was an effect of joint angle ($p < 0.05$). The singlet, doublet and quadruplet at 90 degrees yielded higher activation capacity values than the respective stimuli at 30 degrees (9-18% difference, $p < 0.05$), while the octuplet yielded no difference ($p > 0.05$) between joint angles (Figure 3). The ratio of the resting twitch to the superimposed twitch at each joint angle did not differ between the singlet, doublet, quadruplet and octuplet ($p > 0.05$). RTD for the octuplet did not differ between joint angles ($p > 0.05$), while the singlet, doublet and quadruplet yielded higher values ($p < 0.05$) at 90 than 30 degrees by 111%, 88% and 44%, respectively (Table 1).

The CAR outcome depended on number of stimuli. At the knee joint angle of 30 degrees, the singlet yielded higher activation values than the doublet (4% difference, $p < 0.05$), quadruplet (9% difference, $p < 0.05$) and octuplet (12% difference, $p < 0.05$), and the doublet yielded higher activation values than the quadruplet (6% difference, $p < 0.05$) and octuplet (9% difference, $p < 0.05$). At 90 degrees the singlet produced higher activation than the quadruplet and octuplet (3% and 4% respectively, $p < 0.05$), but no differences were found in the comparisons involving the doublet and between the quadruplet and octuplet ($p > 0.05$). In contrast to the ITT method, there was no effect of joint angle on the CAR method outcome for any number of stimuli ($p > 0.05$). For any given stimuli number and joint angle, the CAR method produced higher activation capacity values by 8-16% than the ITT method. These differences reached statistical significance ($p < 0.05$) for the singlet, doublet and quadruplet at 30 degrees (Figure 3).

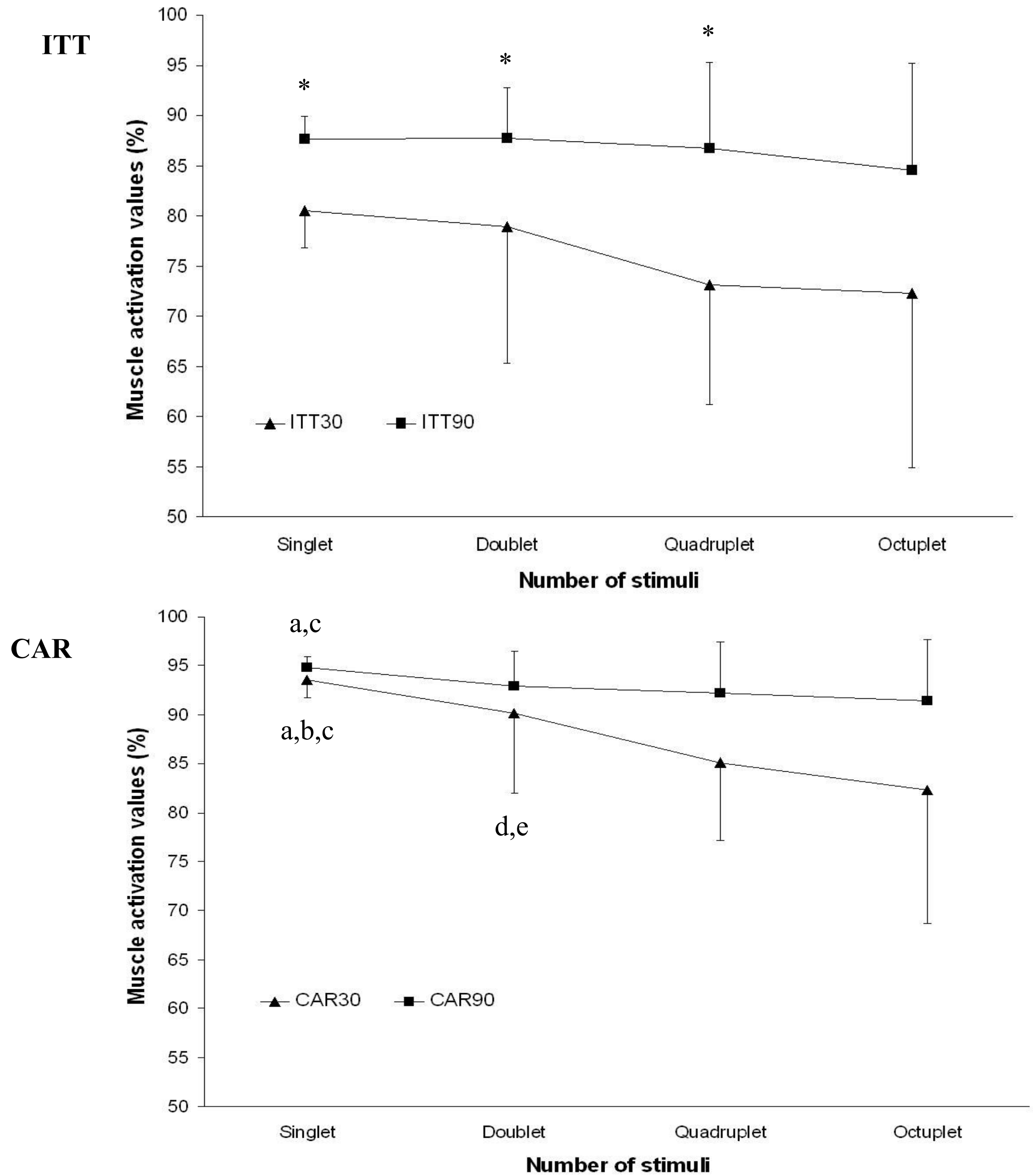

Figure 3. Muscle activation values for the ITT (top panel) and CAR (bottom panel) methods at 30 and 90 degrees knee joint angles using the four different stimuli number in the study. ITT30 and ITT90, activation values at 30 and 90 degrees knee joint angle, respectively, with the ITT method; CAR30 and CAR90, activation

values at 30 and 90 degrees knee joint angle, respectively, with the CAR method. Significant differences are indicated by * between joint angles, *a* between singlet and doublet, *b* between singlet and quadruplet, *c* between singlet and quadruplet, *d* between doublet and quadruplet and *e* between doublet and octuplet. Vertical bars denote SD.

DISCUSSION

The present study manipulated the number of electrical stimuli and the knee joint angle and showed that for a given volitional knee extension effort, a) differences in stimuli number have a greater effect on the CAR than ITT method, and b) knee joint angle changes have a greater effect on the ITT than CAR method. These results support the hypotheses.

The application of a train of maximal intensity stimuli often causes discomfort and limits the applicability of electrical stimulation for the assessment of activation capacity (Dowling et al, 1994; Shield and Zhou, 2004). Nonetheless, it has previously been suggested that multiple stimuli rather than single twitches are required to improve the signal-to-noise ratio and increase the sensitivity of the ITT method by reducing the variability of the superimposed force (Suter and Herzog, 1998) and more effectively overcoming the antidromic effect of stimulation and spinal reflexes (Behm et al, 2001), especially during nerve stimulation. However, increasing the number of stimuli in the present study did not alter the activation values calculated with the ITT method at either knee joint angle. A similar finding has been reported for the level of knee extensor muscle activation obtained by extrapolating the curve describing the relation between the torque ratio of superimposed twitch to resting twitch, and voluntary torque, when using one, two and five stimuli (Behm et al, 1996). Similarly, no differences in elbow flexor activation capacity were found by applying the ITT method using one, two and four stimuli, due to a similarity in the magnitude of the superimposed torque evoked by the three stimulations (Allen et al, 1998). In the present study, as in other studies (Kent-Braun and Le Blanc, 1996; Miller et al, 1999; Strojnik, 1995), the magnitude of the extra torque generated by superimposing current increased with stimuli number (201% and 98% increase from singlet to octuplet at 30 and 90 deg, respectively) indicating fuller activation due to increases in myofibrillar calcium concentration (Ebashi and Endo, 1968; Endo, 1977). However, when this extra torque was normalized to the corresponding torque produced by the reference resting stimulus the differences between stimuli number disappeared, thus producing a constant ITT outcome.

Studies on the effect of joint angle on activation capacity assessed using the ITT method are scarce and report inconsistent results, with longer muscle lengths yielding higher (Kubo et al, 2004), lower (Suter and Herzog, 1997), or similar (Newman et al, 2003) activation values compared with shorter muscle lengths. The current ITT results suggest higher activations at longer lengths when using one, two and four stimuli, and similar activations at shorter and longer lengths when using eight stimuli. In seeking to address whether the inter-angle variation in the ITT outcome in any given study is a true biological effect, the effect of SEC stiffness differences at different muscle lengths should be considered. Changes in SEC stiffness can affect the magnitude of the twitch force (Hill, 1951; Loring and Hershenshon, 1992). One important factor that can affect the SEC stiffness is joint angle. This is supported by ultrasound-based findings that passive joint rotation alters not only muscle fascicle length, but also tendon length and therefore its tensile stiffness (Herbert et al, 2002). At 90 degrees knee angle the quadriceps

SEC would be longer and stiffer than at 30 degrees, thus being able to more faithfully transmit the resting twitch force to the tibia, as evidenced by the differences in the corresponding rate of torque development for the singlet, doublet and quadruplet. Reducing the measurement sensitivity of the ITT method to changes in resting series elasticity would require application of reference forces that can be transmitted equally faithfully across joint angles. The ITT and rate of torque development results indicate that this criterion was met by the application of octuplets.

The present ITT activation capacity values at 30 deg knee flexion are lower than the average ITT values reported for quadriceps voluntary activation, which range from ~84% to 95% (Behm et al, 2001; Behm et al, 2002; Kubo et al, 2004; Newman et al, 2003). However, our ITT values at 90 deg fall within the above range. This difference may be attributed to the fact that the vast majority of published quadriceps ITT values refer to measurements at 90 deg knee joint angle. As explained above, the quadriceps SEC at 90 deg is stiffer than at 30 deg, thus yielding higher resting twitch responses and lower activation estimates.

In agreement with Behm et al (2001), the CAR method yielded higher values than the ITT method. The present difference, however, was found to be joint angle-dependent, reaching the level of 16% at shorter muscle lengths and 8% at longer muscle lengths. Contrary to the similarity in CAR outcome between a doublet and a tetanus reported by Behm et al (2001), CAR in the present study yielded lower values at higher stimuli numbers. The increase in superimposed torque with stimuli number indicates the ineffectiveness of low stimuli number to fully activate muscle fibres left inactivated by volition during MVC and explains the corresponding decreasing activation capacity calculated using the CAR method. However, it must be stressed that percutaneous muscle stimulation can activate only those muscle fibres with nerve endings in the vicinity of the electrodes (Hultman et al, 1983). Direct nerve stimulation is required to evoke the maximum force in all the inactivated muscle fibres during MVC, but this procedure may cause intolerable discomfort raising ethical concerns. In addition, antagonist muscles will co-contract if also innervated by the stimulating nerve, as is the case for the antagonist sartorius muscle during femoral nerve stimulation for quadriceps muscle testing. Nevertheless, the CAR values obtained are likely lower than those that would have been obtained by direct nerve stimulation (Kent-Braun and Le Blanc, 1996). In contrast to the CAR method, experimental findings show that the ITT outcome is largely independent of stimulating site (muscle or nerve), indicating a similarity between protocols in the proportion of activated muscle by superimposed stimulation relative to the stimulation at rest (Rutherford et al, 1986).

Contrary to the ITT method, there was very small variation in the CAR outcome with joint angle for any given number of stimuli, which substantiates our hypothesis. However, the number of stimuli required to obtain the lowest CAR value was joint angle-dependent: While at 30 deg knee joint angle the lowest CAR output (highest superimposed torque) was taken with the octuplet, at 90 deg the quadruplet and octuplet produced similar CAR values. It is likely that the inter-angle difference in the number of stimuli required to obtain the lowest CAR values may have been caused by an increased sensitivity of the submaximally recruited muscle fibres to changes in myofibrillar calcium concentration at longer lengths (for a review see Stephenson & Wendt, 1984).

To conclude, the present results show that the ITT method is more sensitive to changes in joint angle and less sensitive to changes in stimuli number than the CAR method. Based on these findings, it is recommended that for a valid comparison of ITT results between tests corresponding to different SEC stiffness values, a number of stimuli adequate to similarly stretch and stiffen the resting muscle-tendon unit in all tests be delivered. Apart from tests at different muscle lengths, the above recommendation also applies to tests in different age groups (Karamanidis and Arampatzis, 2006; Onambele et al, 2006), and groups with different physical activity histories and lifestyles (Kubo et al, 2000a; Kubo et al, 200b; Maganaris et al, 2006), tests before and after acute (Kubo et al, 2005; Maganaris, 2003) and chronic interventions (Kubo et al, 2002; Reeves et al, 2005; Reeves et al, 2003), and generally in all conditions that may alter the mechanical properties of tendon. Measurements of rate of torque development during stimulation may be used as a guide for assessing whether the criterion of similar passive SEC stiffness between conditions is met, especially when muscle fibre composition is similar. Comparisons of CAR results between tests corresponding to different SEC stiffness values are relatively immune to the above problem. However, to obtain a realistic CAR outcome at a given SEC stiffness state (e.g. a given joint angle in a given population at a given point in time) appropriate steps need to be taken to ensure that a substantial part of the inactivated muscle by volition is activated by the superimposed stimulation.

CHAPTER 4

IS MAXIMUM STIMULATION INTENSITY REQUIRED IN THE ASSESSMENT OF MUSCLE ACTIVATION CAPACITY?

A version of the work from this chapter has been published as:

Bampouras TM, Reeves ND, Baltzopoulos V, Jones D, Maganaris CN. Is maximum stimulation intensity required in the assessment of muscle activation capacity? J Electromyogr Kinesiol 2012; 22(6):873–877. (Appendix 2)

ABSTRACT

Voluntary activation assessment using the interpolation twitch technique (ITT) has almost invariably been applied using maximal stimulation intensity, i.e. an intensity beyond which no additional joint moment or external force is produced by increasing further the intensity of stimulation. The aim of the study was to identify the minimum stimulation intensity at which percutaneous ITT yields valid results. Maximal stimulation intensity and the force produced at that intensity were identified for the quadriceps muscle using percutaneous electrodes in eight active men. The stimulation intensities producing 10 to 90% (in 10% increments) of that force were determined and subsequently applied during isometric contractions at 90% of maximum voluntary contraction (MVC) via twitch doublets. Muscle activation was calculated with the ITT and pain scores were obtained for each stimulation intensity and compared to the respective values at maximum stimulation intensity. Muscle activation at maximal stimulation intensity was 91.6 (2.5)%. The lowest stimulation intensity yielding comparable muscle activation results to maximal stimulation was 50% (88.8 ± 3.9%, $p < 0.05$). Pain score at maximal stimulation intensity was 6.6 ± 1.5 cm and it was significantly reduced at 60% stimulation intensity (3.7 ± 1.5 cm, $p < 0.05$) compared to maximal stimulation intensity. Submaximal stimulation can produce valid ITT results while reducing the discomfort obtained by the subjects, widening the assessment of ITT to situations where discomfort may otherwise impede maximal electrostimulation.

INTRODUCTION

Muscle strength, measured as joint moment or force applied externally during a maximum voluntary contraction (MVC), is determined by a number of factors, including the size of the agonist muscles and their moment arms, the joint angle tested which affects muscle length, the specific tension of the muscle, antagonist muscle co-contraction, and the level of voluntary agonist muscle activation during the test. The assessment of this last factor, voluntary activation, requires the application of artificial stimulation to the muscle and this has been routinely applied in several populations, including children (O'Brien et al, 2010; O'Brien et al, 2008), older individuals (Morse et a,. 2008; Reeves et al, 2003), patients with musculoskeletal disorders (Rutherford et al, 1986; Suter et al, 1998) and in intervention studies involving various types of exercise training (e.g., Knight and Kamen, 2001; Maffiuletti et al, 2000; Selkowitz, 1985) and disuse (e.g., de Boer et al, 2007; Lewek et al, 2001; Sisk et al, 1987).

Voluntary activation is typically assessed with the interpolated twitch technique (ITT; Merton, 1954), according to the equation:

$$\text{Activation level (\%)} = (1 - SI / R) \times 100 \qquad \text{(eq. 1)}$$

where, SI is the additional joint moment produced by superimposing the electrical stimulus on the MVC and R is the joint moment produced by the same stimulus applied at rest. Investigators generally strive to use maximal stimulation for the ITT

(Babault et al, 2003; Behm et al, 2001; De Serres and Enoka, 1998; Kent-Braun and Le-Blanc, 1996; Morse et al, 2008; O'Brien et al, 2008), but there is often some confusion as to what maximality means and whether it is essential for the valid estimation of voluntary activation. To obtain the maximum force from a muscle it is necessary that all motor units are activated and that they are stimulated at frequencies, generally in the order of 30-100 Hz (Gerritts et al, 1999), that generate maximum force. Percutaneous stimulation of a large muscle such as the quadriceps is unlikely to ever activate all motor units. Activation of all motor units can be achieved with direct stimulation of the femoral nerve. Possibly the only time that true maximality of stimulation was achieved during a voluntary contraction was with tetanic stimulation of the femoral nerve with increasing stimulus intensity (Bigland-Ritchie et al, 1978), but this is not a procedure that is well tolerated by most subjects. Irrespective of whether all motor units are activated, it is very unlikely that they will be producing their maximum force since most ITT tests involve using twitches or doublets rather than tetanic trains.

One issue associated with using twitches or doublets to stimulate the resting muscle is that the relatively small and transitory forces will be recorded as smaller tension transients due to stretching of the series elastic components of the apparatus and the muscle-tendon unit. When superimposed on a voluntary contraction where the series elements are already stretched the tension transient will more faithfully reflect the force produced by the muscle. This will tend to increase the SI/R ratio and thus give a false low value for voluntary activation. One way of reducing the series compliance of the quadriceps is to flex the knee, in order to increase the muscle-tendon unit stiffness.

Another possible way of avoiding the problems associated with comparing twitches of resting with active muscle is by using the Central Activation Ratio (CAR) which only depends on the superimposed force or joint moment during MVC and not the stimulation at rest (CAR = MVC/(MVC+SI)). However, it is very unlikely that the superimposed stimulation will maximally activate all the muscle.

The question is therefore how much of the muscle needs to be activated to achieve a valid answer using the ITT. Behm et al (1996) and de Ruiter et al (2004) suggest that it is necessary to stimulate nearly all the muscle. However, Rutherford et al (1986) compared femoral nerve stimulation, which was assumed to activate all motor units, and percutaneous quadriceps muscle stimulation that activated only a portion of the muscle, and found no differences in the SI/R ratio between the two stimulation modes. This notion was supported by Newman et al (2003), who compared magnetic (with the coil positioned on the femoral nerve) and percutaneous muscle stimulation (with electrodes on the distal and proximal end of the quadriceps) and found no difference between the two stimulation techniques, suggesting that stimulating a portion of the muscle yields similar activation results to stimulating the whole of the muscle. When using percutaneous stimulation, Rutherford et al (1986) stated that they adjusted the stimulus intensity used for the superimposed twitches in relation to the proportion of the MVC force generated when stimulating at 30 Hz. However, they did not specify what that force was nor present any evidence as to what the minimum required force might be.

Consequently, the aim of the present study was to identify the minimum stimulation intensity at which muscle activation could be validly assessed, reducing the discomfort associated with high intensity stimulation and thus widening the applicability of ITT assessment to a greater range of subjects and patients.

METHODS

Eight healthy, physically active men (mean ± SD: age 28.9 ± 5.0 years, height 1.80 ± 0.09 m, body mass 83.9 ± 15.3 kg) volunteered to participate in the study. To ensure consistency in performance, all subjects were familiar with the experimental procedures involved (Button and Behm, 2008) and were tested in the laboratory on a single occasion.

Ethical approval for the study was granted by the Ethics Committee of the Institute for Biomedical Research into Human Movement and Health of Manchester Metropolitan University, UK. All subjects provided written informed consent prior to any testing. The study complied with the Declaration of Helsinki.

The mechanical output of isometric knee extension was measured as force applied externally in the sagittal plane at the level of the ankle, at right angles to the longitudinal axis of the lower leg. The subjects sat in the chair of a custom-made dynamometer (de Ruiter et al, 2004; Kooistra et al, 2007), with the hip joint angle at 85^o (supine position = 0^o) and the right leg at a knee joint angle of 90^o (full knee extension = 0^o). Straps were positioned over the hips and tested thigh to prevent extraneous movement and the lower leg was securely strapped to a force-transducer (KAP, E/200 Hz, Bienfait B.V. Haarlem, The Netherlands) at the ankle. Force signals were corrected for passive tension of the knee extensors and real-time force readings were displayed online and recorded for further analysis (Matlab, The Mathworks, Natick, MA).

Two 7 x 12.5-cm self-adhesive carbon rubber electrodes (Versa-Stim, ConMed, New York, USA) were placed on the proximal and distal regions of the quadriceps muscle group with the cathode being the proximal electrode. Their placement was determined by the position that generated the highest possible knee extension force when stimulated by a twitch. Stimuli of 200-μs pulse width and 10-ms inter-stimulus gap were generated by an electrical stimulator (model DS7, Digitimer stimulator, Welwyn, Garden City, UK) modified to deliver a maximum of 1,000 mA output. Electrical stimuli application was displayed online along with the force signal.

Maximal stimulation intensity was determined by application of single twitches at rest, with the voltage set at 300 V and the current intensity increasing by 50 mA for each application. Maximal stimulation intensity (hereafter called the maximal intensity) was determined as that beyond which a further increase in current by 50 mA failed to increase the twitch force further.

The stimulation intensities required to produce 10 to 100% (in 10% increments) of the force at maximal intensity were determined in a randomized order. Typically, this procedure required application of 2-3 twitches at each percentage of the maximal intensity to identify the appropriate current. Duration of rest between stimuli applications was 2-3 min. These stimulation intensities were then used for the rest of the experiment (hereafter called percentage intensities).

Subjects performed an MVC and all subsequent test contractions were performed at 90% of MVC. This contraction level was selected as our laboratory and others have found it to be a near-maximal contraction level that subjects can achieve consistently (Behm et al, 1996; Bülow et al, 1993). A target line indicating 90% of MVC was displayed on the same screen as the force from which the subjects received visual feedback to help them maintain a steady and consistent force.

The subjects were required to perform 9 trials at 90% of MVC with 3-4 min rest interval. Typically, these trials lasted ~2 s. During each trial, two stimuli (doublet) were applied as soon as a force plateau occurred (determined visually) while a second doublet was applied exactly three seconds later, during complete relaxation (resting doublet). The doublet was selected over a higher number of stimuli based on previous experimentation finding no differences between a doublet and a quadruplet or an octuplet on the ITT value for the quadriceps muscle. The ITT (eq.1) value for each percentage intensity was calculated.

To assess the level of discomfort associated with a given percentage intensity, an unmarked 10 cm visual analog pain intensity scale (VAS, Collins et al, 1997), with ‘No pain’ at one end and ‘Worst pain’ at the other end, was used to record the level of discomfort experienced by the subjects after each stimulus intensity. Scores above 5.4 cm indicate severe pain, while scores above 3 cm indicate moderate pain (Collins et al, 1997).

Normality of data was examined using the Shapiro-Wilk test and was subsequently confirmed for all variables (90% MVC, activation level, VAS pain scores). A repeated measures analysis of variance was used to ascertain comparability of 90% MVC force across the trials with the different percentage intensities.

Differences between percentage intensities and maximal intensity for activation level and VAS scores were examined using Dunnett's test. This test is more appropriate in situations where several treatments are to be compared against a control or reference treatment only, rather than comparisons between all treatments (Dunnett, 1955). The smallest percentage intensity for which muscle activation did not differ significantly from that of the maximal intensity was considered to be the minimum intensity able to yield valid results. Significance was set at $p < 0.05$. Values are presented as mean ± SD, unless otherwise indicated.

RESULTS

The subjects' MVC force was 748 ± 130 N. The 90% MVC force was not significantly different ($p = 0.477$) between the trials with the different percentage intensities (Table 2) and demonstrated low variability (coefficient of variation 2.5 (1.2) %). The resting stimulus force at maximal intensity was 302 ± 62 N (Figure 4).

Table 2. 90% of MVC force values and VAS pain scores for each percentage intensity. Data are presented as mean ± SD. * indicates significant difference between a given percentage intensity and the maximal intensity.

Percentage intensity (%)	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
90% MVC (N)	639 ± 102	643 ± 110	631 ± 119	631 ± 116	628 ± 114	641 ± 108	626 ± 106	639 ± 108	634 ± 115	636 ± 117
VAS (cm)	1.5 ± 1.9*	1.4 ± 1.3*	1.6 ± 1.2*	3.0 ± 1.7*	4.0 ± 2.2*	3.7 ± 1.5*	4.4 ± 1.7	4.9 ± 2.3	6.4 ± 2.5	6.6 ± 1.5

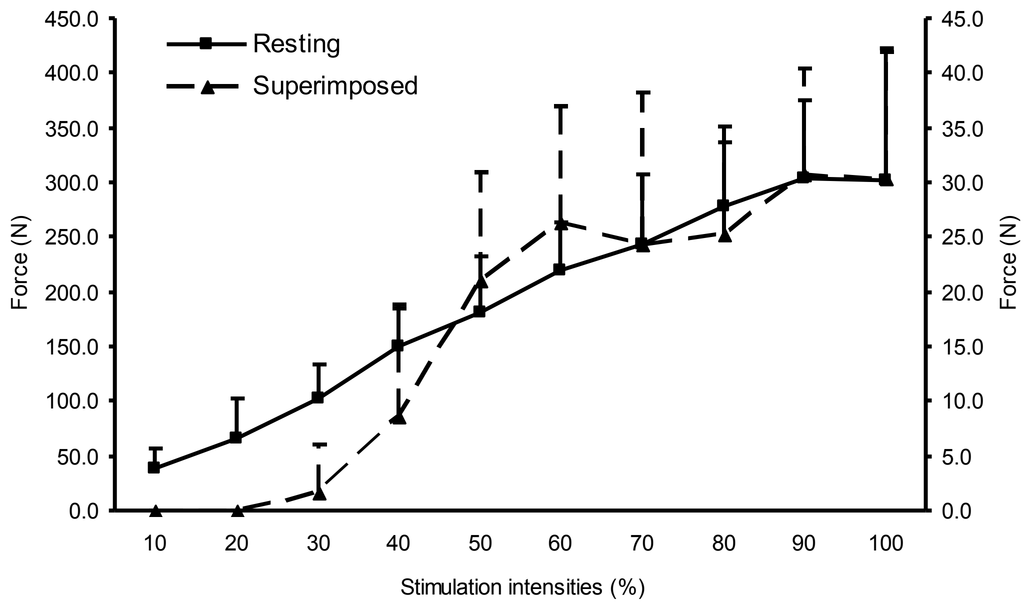

Figure 4. Mean superimposed (left y axis) and resting (right y axis) doublet magnitudes across all percentage intensities. Vertical bars denote SD.

Muscle activation at maximal stimulation intensity was 91.6 ± 2.5%. Percentage intensities of 90-50% yielded similar muscle activation values compared to the maximal intensity ($p > 0.05$). However, the percentage intensities of 40-10% produced significantly different muscle activation values ($p < 0.05$) than maximal intensity. Therefore, 50% of maximal intensity was the mean lowest percentage intensity yielding a valid ITT outcome (muscle activation 88.8 ± 3.9 %) (Figure 5).

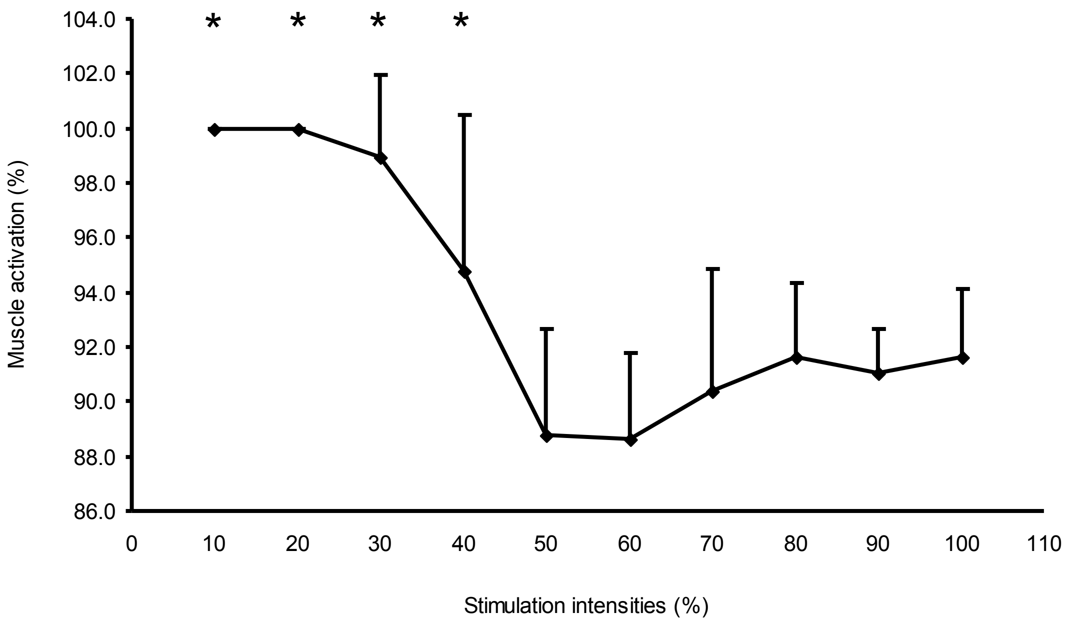

Figure 5. Mean muscle activation values across all percentage intensities. Vertical bars denote SD. * indicates significant difference ($p < 0.05$) compared to maximal intensity.

However, visual inspection of individual graphs indicated that in some subjects a valid ITT outcome could be obtained with intensities around 30% of maximal intensity (Figure 6).

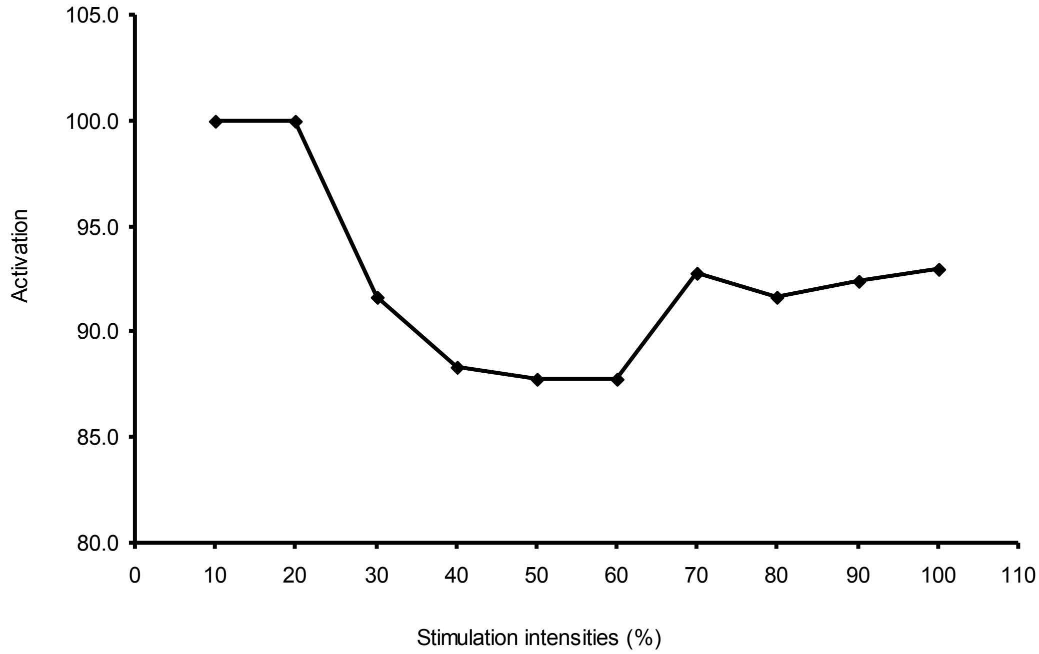

Figure 6. Muscle activation values across all percentage intensities for a single subject, showing a plateau in muscle activation occurring below 30% percentage intensity.

VAS indicated that pain at percentage intensities of 90-70% was similar to the pain experienced at maximal intensity. However, pain at 60-10% stimulation intensities was significantly lower ($p < 0.05$). The pain scores were reduced from 6.6 ± 1.5 cm at maximal intensity to 3.7 ± 1.5 cm at 60% percentage intensity (Table 2).

DISCUSSION

The aim of the study was to identify the minimum stimulation intensity that could yield valid muscle activation values, similar to those obtained with maximal intensity. It was found that stimulation at 50% of maximal intensity is sufficient to obtain a valid ITT outcome. The discomfort experienced by the subjects at this stimulation intensity was also reduced from severe to moderate compared to maximal stimulation.

Many previous authors have used what they term “maximal” stimulation intensities in an attempt to activate the largest portion of muscle possible and avoid erroneous ITT estimates (Behm et al, 2001; de Ruiter et al, 2004; Kent-Braun and Le-Blanc, 1996; Knight and Kamen, 2001; Kooistra et al, 2007; Morse et al, 2008; O’Brien et al, 2008; Reeves et al, 2003). However, a comparison between percutaneous muscle stimulation, which only activates a proportion of the muscle, and nerve stimulation, which activates all the motor units (Rutherford et al, 1986), showed no differences in the ITT outcome between the two techniques, suggesting that valid results can be achieved as long as the portion of the muscle activated at rest and during contraction remains the same. The findings of the current study support those of Rutherford et al (1986), indicating that valid ITT results can be obtained even when activating relatively small portions of the quadriceps muscle.

The mechanisms underlying the pattern of the ITT and the results obtained in the present study can be better understood by considering the changes with percentage intensity and the magnitude of the corresponding mean values of the superimposed and resting doublets independently (Figure 4). At lower stimulation intensities (10% and 20% of maximal intensity), a very small proportion of inactive muscle would become activated by the superimposed doublet. Although this stimulation intensity suffices to produce a detectable force increment when the doublet is applied at rest, it is difficult to detect the superimposed doublet since any force increment is small in relation to the oscillation of the voluntary force trace. This results in zero SI/R ratios and a misleading conclusion of complete activation. At 30% and 40% of maximal intensity a larger portion of muscle becomes activated and the magnitude of the superimposed doublet increases rapidly. Following that point the stimulation intensity reaches a level that is sufficiently high to induce both detectable increases in the superimposed stimulus as well as sufficiently stretch the series elastic components at rest, resulting in a constant SI/R ratio and, thus, in valid ITT results.

Maximal stimulation is an imprecise term since it can vary with the type of stimulator, the type, size and position of the electrodes as well as the conductivity of the skin and subcutaneous fat and the size of the muscle. It is therefore more useful to define the minimum requirements for testing activation in terms of the force generated by the electrical stimulation as a percentage of the likely MVC force. In the subjects participating in the current study, the mean 90% of MVC was 635 N and valid estimates of ITT were obtained with a percentage intensity that generated a mean force of 181 N in the resting muscle. Consequently, it is recommended that the stimulation intensity should be set to generate at least one third of the estimated MVC.

A concern with electrical stimulation is sometimes the discomfort experienced by subjects (Behm et al, 2001; Chae et al, 1998; Delitto et al, 1992; Han et al, 2006; Miller et al, 2003; Valli et al, 2002). Two studies have indicated high levels of discomfort in older subjects (Valli et al, 2003) and patients (Chae et al, 1998), subject groups where it is particularly important to assess the ability to activate their muscles (Chae et al, 1998). Subject discomfort was investigated by Miller et al (2003) by inducing pulse trains of different lengths and durations. Less discomfort was reported with shorter pulse durations without a change in the activation results. Suggestions were made for more research into protocols that can assess muscle activation validly, with reduced discomfort of the subjects. The present findings suggest that discomfort was significantly reduced at percentage intensities below 70%. The average difference in VAS scores was reduced by 2.9 cm. Previous studies suggested 2 cm as the minimum clinically significant change when using VAS (DeLoach et al, 1998). Therefore, the present results indicate a reduction from severe to moderate pain, which is important because it widens the applicability of ITT assessment to subjects who are less tolerant of high intensity stimulation, thus reducing potential drop-out and addressing ethical concerns.

Another potential problem with the application of transcutaneous electrical stimulation for assessing activation capacity using the ITT method is co-contraction of: a) nearby agonist muscles due to current spread (Taylor, 2009), b) antagonist muscles due to activation of cutaneous receptors (Belanger and McComas, 1981; Poumarat et al, 1991) and c) antagonist muscles due to discomfort (Paillard et al, 2005). The latter effect will be less of a problem with submaximal stimulation. Nevertheless, electromyography can be used to detect any artefactual co-contractions from non-studied muscles and make appropriate relevant adjustments (e.g., alter size or position of stimulating electrodes).

In conclusion, this study shows that maximal stimulation is not necessary to obtain a valid ITT outcome. The results for the knee extensor muscles of healthy young adults show that valid ITT results for contractions at 90% of MVC can be obtained with just 50% of maximal intensity. Practically, a more useful guide is that the force generated by stimulation of the resting muscle should be at least one third of the anticipated MVC force.

CHAPTER 5

THE ROLE OF AGONIST AND ANTAGONIST MUSCLES IN EXPLAINING THE VARIATION IN ISOMETRIC KNEE EXTENSION TORQUE WITH HIP JOINT ANGLE

A version of the work from this chapter is under review for publication in the Scandinavian Journal of Medicine and Science in Sports.

ABSTRACT

Musculoskeletal modelling studies predict higher quadriceps torque in supine (longer rectus femoris) compared to seated (shorter rectus femoris) position, contradicted by experimental studies, typically utilising voluntary contractions. Incomplete muscle activation in the supine position has been proposed as the reason for this discrepancy, but differences in antagonistic co-activation could also be responsible due to altered hamstrings length. The study examined the role of agonist and antagonist muscles in explaining the variation in isometric knee extension torque with changes in hip joint angle. Knee extension torque was recorded during maximum voluntary isometric contractions (joint MVC) in seated and supine positions. Antagonistic co-activation torque was estimated and added to the respective joint MVC (corrected MVC). Quadriceps torque was also recorded from submaximal tetanic stimulation. Joint MVC was not different ($p > 0.05$) between supine (245 ± 71.8 Nm) and seated (241 ± 69.8 Nm) positions and neither was corrected MVC (257 ± 77.7 Nm and 267±87.0 Nm, respectively). Antagonistic torque was higher ($p = 0.025$) in the seated (26 ± 20.4 Nm) than in the supine position (12 ± 7.4 Nm). Tetanic torque was higher ($p = 0.001$) in the supine (111 ± 31.9 Nm) than in the seated (99 ± 27.5 Nm) position. The similar joint MVC torque and higher tetanic torque confirm previous discrepancies between experimental and modelling studies. The lower estimated antagonistic torque when supine, suggests that antagonistic co-activation does not contribute to these discrepancies. Lower quadriceps activation in the supine position may be due to inadequate pelvis fixation and/or to reduced vestibular feedback. The study is unique in examining the potential mechanisms explaining the discrepancies and can assist standardising muscle function assessment and development of more accurate muscle models.

INTRODUCTION

Isometric knee extensor muscle torque changes with hip joint angle (e.g. Maffiuletti and Lepers, 2003; Rochette et al, 2003). This is because hip joint angle impacts on the length of the biarticular rectus femoris muscle, which has been estimated to contribute up to ~17% to the quadriceps torque output (McNair et al, 1991).

A striking discrepancy, however, exists, in the direction of knee extensor muscle torque change with hip joint angle between experimental and musculoskeletal modelling studies (Herzog et al, 1991). Experimental studies have reported higher torque at the seated (shortened rectus femoris muscle) versus the supine (lengthened rectus femoris muscle) positions (e.g. Maffiuletti and Lepers, 2003; Rochette et al, 2003). In contrast, musculoskeletal modelling studies determining in vivo the excursion range of the rectus femoris have shown this muscle to operate on the ascending limb of the force-length curve (Herzog and ter Keurs, 1988). Thus, higher quadriceps torque would be expected with the rectus femoris muscle in the lengthened rather than the shortened position (Herzog and ter Keurs, 1988; Hoy et al, 1990; Lewis et al, 2009).

Since experimental studies typically use voluntary contractions, it can be hypothesised that the discrepancy might be due to altered rectus femoris muscle activation at the different muscle lengths (Herzog et al, 1991). This notion was supported by Maffiuletti and Lepers (2003), who examined quadriceps activation in the seated and supine positions. Stimulating the femoral nerve to quantify quadriceps activation, they reported a significant 4.2% higher quadriceps activation when in seated position compared to supine. This finding supported the notion that muscle activation is one reason for the discrepancy between experimental and musculoskeletal studies.

A second, and currently unexplored, reason for the lack of agreement between experimental and modelling studies could be differences in antagonistic muscle co-activation between the different hip joint angles. Joint torque generated during knee extension is the 'net' sum of agonistic muscles positive moment and the antagonistic knee flexor muscles negative moment (Kubo et al, 2004). A change in hip joint angle will result not only in a change of the rectus femoris length but also in a change of the hamstrings length. In isometric knee extension experiments where the hip joint angle was kept constant and the hamstrings length was manipulated by changing the knee joint angle, hamstrings co-activation increased at more knee flexed positions (i.e. shorter hamstring muscle-tendon unit length) (Kubo et al, 2004). Based on these results, shorter hamstrings muscle-tendon unit length, caused by extending the hip (rather than flexing the knee), could also increase co-activation of the hamstrings, subsequently increasing antagonistic torque and ultimately affecting the measured net isometric knee extension torque.

To examine whether antagonistic co-activation contributes to the discrepancy between experimental and musculoskeletal studies, the present study aimed to quantify and compare agonist activation and antagonistic co-activation in the seated and supine positions. Electrical stimulation of the quadriceps muscle was utilised to allow examination of muscle function bypassing the subjects' voluntary neural input. It was hypothesised that increased hamstring co-activation would be found in the supine position, thus partly explaining why contrary to modelling predictions knee extension torque is lower when the hip is extended.

METHODS

The study complied with the Declaration of Helsinki and the study and the procedures followed were approved by the Institutional Ethics Committee. Nine healthy, active males (age 30.2 ± 7.7 years, stature 1.78 ± 0.09 m, body mass 81.7 ± 11.2 kg) free from any musculoskeletal injuries gave written, informed consent to participate in the study. In order to reduce variability in performance, all subjects were familiar with the experimental procedures (Button and Behm, 2008) and all testing took place on a single testing session.

The subjects were tested in two positions, seated (hip joint angle = 90°), and supine (hip joint angle 160°) (full hip joint extension = 180°), with the knee and ankle joint angles at 90° for both conditions. A custom-made dynamometer was used for the study. The dynamometer was specifically developed for assessing isometric contractions and as such, the lever arm and the bed had very limited compliance while the restraints allowed for a better fixation of the pelvis and the body during the supine position. For the seated position, the subject sat in the chair of the dynamometer and straps were positioned over the pelvis and tested thigh, to prevent extraneous movement, while the lower leg was securely strapped, above the lateral malleolus, to a force-transducer (KAP, E/200 Hz, Bienfait B.V. Haarlem, The Netherlands). For the supine position, the subject lay in the chair of the dynamometer and the lower leg was securely strapped to the force-transducer above the lateral malleolus while straps were positioned over the pelvis and tested thigh. Pilot testing indicated extraneous movement occurred with the upper body moving upwards along the dynamometer's backrest. Hence, to prevent this movement, mechanical blocks were fixed securely in place in contact with the shoulders, which held the body in position without allowing any upwards movement (Figure 7).

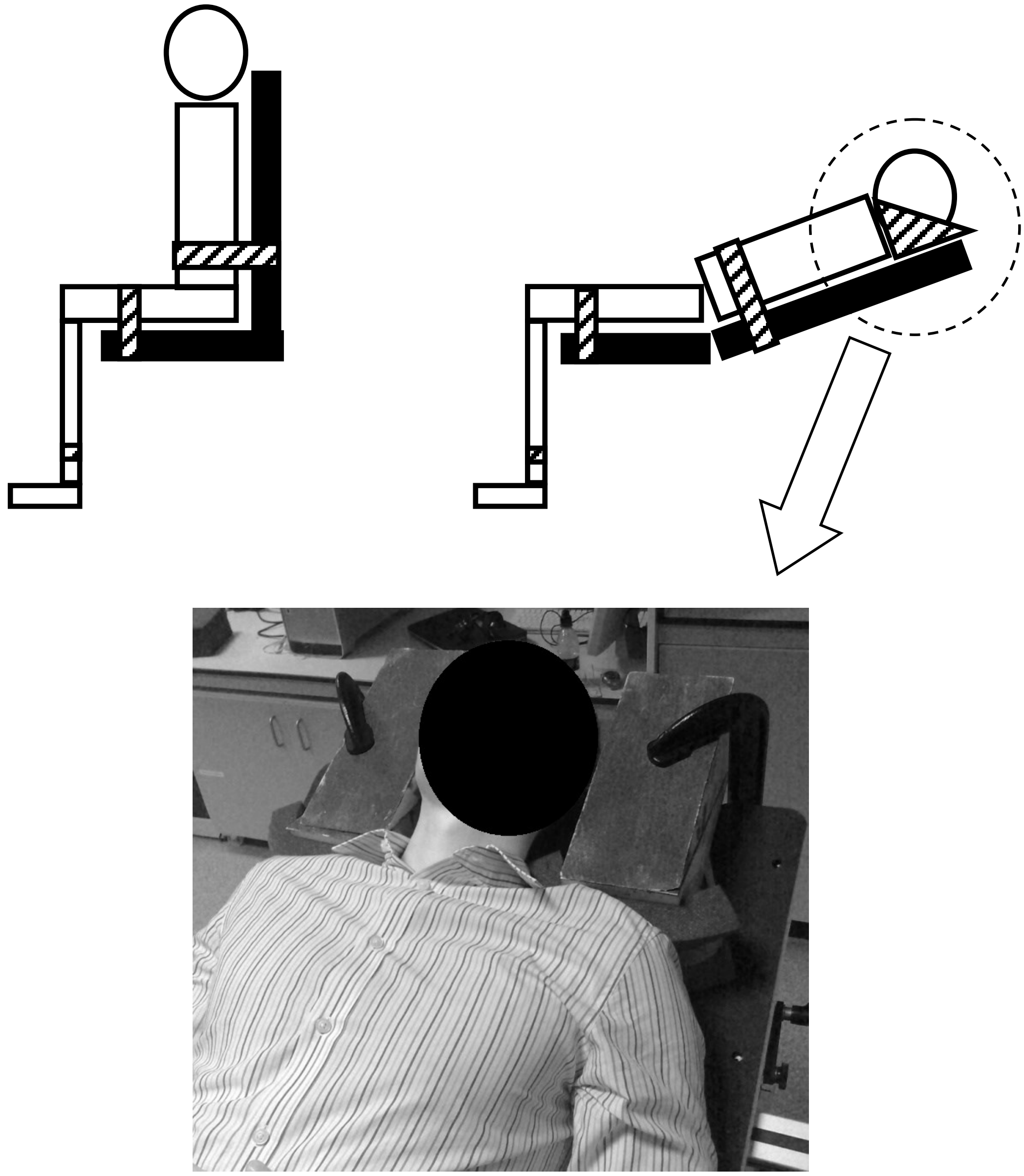

Figure 7. Schematic diagram of the experimental set up (top) for seated (left) and supine position (right, 160° hip joint angle, 180° = full extension). Solid rectangular shapes represent the dynamometer seat. Rectangular patterned shapes indicate straps placed on the lower leg (securely strapped to a force transducer), tested thigh and pelvis, for both conditions. Triangular patterned shapes indicate mechanical blocks used in the supine position to avoid extraneous movement upwards. Specific details of these blocks can be seen at the picture (bottom).

Subsequent testing confirmed that this set-up kept extraneous movement to an absolute minimum and was superior compared to our experience with other commercially available isokinetic dynamometers.

Force signals were corrected for passive tension of the knee extensors and real-time force readings were displayed online and recorded for further analysis (Matlab, The Mathworks, Natick, MA). The mechanical output of the isometric knee extension was measured as the force applied externally in the sagittal plane at the level of the ankle (at right angles to the longitudinal axis of the lower leg), and converted to torque by multiplying that force by the external moment arm length, which was defined as the distance between the point of the external force application and the knee joint centre.

Two 7 x 12.5-cm self-adhesive carbon rubber electrodes (Versa-Stim, ConMed, New York, USA) were placed on the proximal and distal regions of the quadriceps muscle group with the cathode being the proximal electrode. Stimuli of 200-μs pulse width and 10-ms inter-stimulus gap were generated by an electrical stimulator (model DS7, Digitimer stimulator, Welwyn, Garden City, UK) and applied for a duration of 1s. Electrical stimuli application was displayed online along with the force signal. Percutaneous stimulation was selected over nerve stimulation, as the outcome between the two is comparable (Rutherford et al, 1986) and it reduces the discomfort induced to the subjects (Delitto et al, 1992).

To obtain a baseline of each subject's strength, two isometric knee extension maximum voluntary contractions (joint MVC) were performed and averaged. If the coefficient of variation (calculated as standard deviation / average * 100) between the two joint MVC's was >5% (Kooistra et al, 2007), a third trial was performed and the closest two were averaged.

Supramaximal electrical stimulation intensity was examined in both positions to confirm that the same level of stimulation intensity could activate the muscles to respectively similar (maximal) levels with the rectus femoris lengthened and shortened. Each subject's supramaximal stimulation intensity was determined by the application at rest of single twitches of 200-μs duration, with the voltage set at 300 V and the current intensity increasing by 50 mA for each twitch application. The stimulation intensity that did not elicit any further increase in force output, despite an increase in current by 50 mA, was determined as supramaximal stimulation intensity and subsequently used. For eight subjects, the supramaximal intensity was identical between seated and supine positions, while for one subject the seated position required one additional increment of 50 mA and that was used as the supramaximal intensity for that subject in that position.

Following confirmation of the supramaximal stimulation intensity in both positions, a tetanus of 100Hz, duration of 1 s and of intensity sufficient to yield a force equivalent to one third of the seated MVC was delivered to the muscle at rest, to effectively 'standardise' the muscle's force output in the absence of any voluntary neural input and examine the true influence of rectus femoris muscle length changes on knee extensor torque output. This intensity was selected as appropriate to sufficiently stimulate the muscle without the negative risks associated with maximal tetanic stimulation (Belanger and McComas, 1981). The same tetanic stimulation intensity was used for both positions.

For electromyography (EMG) measurements, two surface Ag-AgCl electrodes of 10mm diameter each, were placed on the long head of the biceps femoris muscle. The electrodes were placed in a bipolar configuration and with a centre-to-centre distance of 20mm, preceded by shaving and cleansing of the placement area. EMG signal was collected at a sampling rate of 1000Hz, and filtered with a high- and low-pass filter of 10 and 500Hz, respectively.

Antagonistic biceps femoris (BF) muscle torque was estimated with the use of EMG (Maganaris et al, 1998; Kubo et al, 2004; Reeves et al, 2004), based on the BF muscle activation when acting as an agonist. Subjects performed four submaximal isometric knee flexions, with BF muscle torque and EMG recorded. Torque was plotted against the EMG signal and a line was fitted through these five data points to provide a regression equation; a minimum coefficient of determination value of 0.9 was set as the fit acceptance criterion. Using the regression equation obtained, the torque contribution from the co-activation of the knee flexor muscles was estimated from the EMG activity. This antagonistic torque was then added to the measured knee extension MVC torque to obtain the ‘corrected MVC’.

The above procedures were followed for both hip joint positions. All trials had adequate rest between them. All measurements took place on the right leg. The initial MVCs were always performed in the seated position, but the order of the rest of the procedures was randomised.

Normality of distribution of the data was checked for and subsequently confirmed using Shapiro-Wilk test. Differences between the two positions for all variables were examined using a dependent Student's t-test. Effect size (ES) was calculated for significantly different comparisons to provide an indication of the magnitude of the effect, with 0.8, 0.5 and 0.2 representing large, moderate and small effects (Fritz et al, 2012). For all statistical analysis IBM SPSS Statistics v19 was used. Data are presented as means ± SD. Statistical significance level was set at $p < 0.05$.

RESULTS

The results showed that knee extension joint MVC was not significantly different between seated (shortened rectus femoris muscle) and supine (lengthened rectus femoris muscle) positions, while tetanic stimulation followed modelling predictions, with supine joint MVC torque being higher than seated ($p = 0.001$). The joint MVC results agree with previous experimental studies (e.g. Maffiuletti and Lepers, 2003; Rochette et al, 2003), while the tetanic stimulation results agree with modelling predictions (Herzog and ter Keurs, 1988; Hoy et al, 1990; Lewis et al, 2009). The antagonistic torque was significantly different ($p = 0.025$) between positions, however in a direction contrary to the study's hypothesis, as the seated position had higher antagonistic torque. Further, when the antagonistic torque was accounted for, the corrected MVC still did not follow the same pattern as the tetanic stimulation, as there was no difference between the two positions, suggesting that antagonistic muscle co-activation was not responsible for the observed experimental results.

Mean supramaximal stimulation intensity was identical between seated and supine positions (512 ± 124.6 mA for both positions), suggesting similar portion of the muscle was activated. Descriptive statistics for joint MVC, tetanic, antagonistic and corrected MVC torque can be seen in Table 3.

Table 3. Descriptive statistics for maximum voluntary contraction (MVC) torque, tetanic stimulation torque, antagonistic torque and corrected MVC for both conditions (seated and supine). Data are presented as mean ± SD. * denotes significant difference at $p < 0.05$ between seated and supine conditions. Effect size (ES) in brackets.

	Joint MVC torque (N•m)	Tetanic torque (N•m)	Antagonistic torque (N•m)	Corrected MVC torque (N•m)
Seated	241 ± 69.8	99 ± 27.5	26 ± 20.4	267 ± 87.0
Supine	245 ± 71.8	111 ± 31.9* (1.0)	12 ± 7.4* (1.0)	257 ± 77.7

DISCUSSION

The aim of the present study was to determine whether antagonistic co-contraction contributes to the discrepancy between experimental and theoretical quantifications of isometric knee extension torque when the rectus femoris length is altered, by manipulating the hip joint angle. The results suggest that differences in antagonistic knee flexor co-activation with hip joint angle do not contribute to this discrepancy. Rather, a reduced voluntary knee extensor muscle activation in the supine position is the major reason for the lower torque when the rectus femoris is in a lengthened position (hip extended), despite this muscle operating on the ascending arm of the force-length relationship (Herzog and ter Keurs, 1988).

Contrary to the study's hypothesis that the antagonist muscles would co-contract more in the supine position to stabilise the pelvis, but also contrary to the findings of Kubo et al (2004), antagonistic co-activation torque was higher at longer biceps femoris muscle lengths (seated position). Further, despite the significant difference in antagonistic co-activation torque between the two positions, corrected MVC (i.e. with the antagonistic torque accounted for) was not significantly different between positions, indicating that antagonistic co-activation cannot constitute a reason for the discrepancy between model predictions of isometric knee torque and experimental observations. The most likely explanation for the reduced antagonistic co-activation in the supine position lies in the common drive hypothesis (Basmajian and De Luca, 1981). The agonist quadriceps and antagonist hamstrings have a common central motor drive, meaning that quadriceps activation and hamstrings co-activation will change concurrently. As the quadriceps muscle demonstrated lower activation in the supine position, antagonistic co-activation would also have to be lower, which concurs with our findings.

The tetanic stimulation results confirm biomechanical models predicting higher isometric knee extension torque in the supine (lengthened rectus femoris) position compared to the seated (shorter rectus femoris muscle) position (Herzog and ter Keurs, 1988; Herzog et al, 1990; Hoy et al, 1990; Lewis et al, 2009). As the rectus femoris muscle operates on the ascending limb of the force-length curve, elongating it would force it to operate closer to, or on the plateau region, generating higher forces and consequently, so does the quadriceps muscle.

Previous experimental studies, however, reported higher MVC torque at the seated position (Maffiuletti and Lepers, 2003; Rochette et al, 2003). These studies utilised maximum voluntary contractions and the MVC output reflects both the mechanical behaviour and voluntary activation capacity of the agonist muscles, but also the level of antagonist muscle co-contraction. Maffiuletti and Lepers (2003) reported higher isometric MVC quadriceps torque by approximately 10%, with a respective increase in activation of 4.2% in the seated position compared to supine, suggesting that increased agonist activation levels at that position resulted in the increased torque. The results from the present study largely agree with the above studies. When the subjects contracted their knee extensors voluntarily, the difference that existed between hip joint positions during tetanic stimulation disappeared, suggesting higher activation in the seated position.

The extended hip joint position, which corresponded to the lower antagonistic co-activation, was also the position with the lower agonist activation. It seems, therefore, that the reduction in agonist activation in the supine position is independent of reciprocal inhibition mechanisms (Tyler and Hutton, 1989), possibly due to inadequate pelvic fixation. Studies typically stabilise the subject by straps placed over shoulders and / or abdomen, to prevent extraneous movement (Hart et al, 1984; Magnusson et al, 1993). These experimental set-ups, however, are unlikely to ensure adequate pelvis stabilisation in the supine position, where the direction of force applied pushes the body in a different direction to the one the straps are counteracting and can negatively impact on the subject's ability to exert maximal volitional effort (Hart et al, 1984; Magnusson et al, 1993). With reduced volitional effort, there would be a concurrent reduction in both agonist and antagonist muscle activation in line with the common drive hypothesis described above.

Another possible reason for the difference in agonist muscle activation between the two hip joint positions could be differences in vestibular feedback. Lewek et al (2006) examined the effect of hip (afferent feedback) and head (vestibular feedback) position on quadriceps EMG during an isometric knee extension MVC, by altering the hip joint angle on its own as well as in combination with the head orientation. When the head position followed the hip position (head in alignment with upper body), there was significantly higher quadriceps EMG activity in seated position compared to supine. This pattern was not seen when the head orientation (vertical to the horizontal) was maintained the same for all hip positions, suggesting that vestibular, and not afferent input, was the prime reason for the change in EMG activity between positions (Lewek et al, 2006). In the present study, the subjects maintained their head in alignment to the body (Figure 7) in both positions, which is likely to have resulted in reduced quadriceps activation in the supine position.

One possible methodological consideration with the present study is the use of percutaneous stimulation. The electrode fixed placement over the muscle belly presents a possible limitation in that moving from the seated to the supine position, a different portion of the rectus femoris muscle may have been stimulated, which could have affected the results (Newman et al, 2003). However, the size of the electrodes we used was large, selected for increased comfort and for enabling stronger contraction (Alon, 1985), and, importantly, covered a wide quadriceps area. Thus, it is unlikely that a significantly different portion of the rectus femoris would have been stimulated.

Another issue with percutaneous stimulation is possible current overflow, which could induce antagonistic co-contraction (Alon et al, 1994). However, the overflow should be of sufficient level to increase activation of the muscle by at least 5% before any effect on torque takes place (De Serres and Enoka, 1998). Given that during joint MVC antagonistic co-contraction was ~5-10% of the agonistic torque, any antagonistic co-contraction induced by current overflow would have been even less (as a percentage of the tetanic stimulation torque) and thus, of negligible impact on the outcome.

The present study shows that although antagonistic hamstrings co-activation torque is substantial and affects the estimation of knee extensor MVC, it cannot explain why contrary to modelling predictions knee extensor MVC is lower when the hip joint is extended. This discrepancy is explained, however, by the lower activation capacity in the supine position. Biomechanical models must consider variations with knee and hip joint positioning in both agonist activation and antagonist co-activation to more accurately reflect experimental observations of quadriceps muscle function.

CHAPTER 6

INTERPLAY BETWEEN BODY STABILISATION AND QUADRICEPS MUSCLE ACTIVATION CAPACITY

A version of the work from this chapter is under review for publication in the Journal of Electromyography and Kinesiology.

ABSTRACT

The study aimed to distinguish between the effect of stabilisation and muscle activation on quadriceps maximal isometric voluntary contraction (MVC) torque generation. Nine subjects performed a) an MVC with restrained leg and pelvis (Typical MVC), b) a Typical MVC with maximal handgrip (Handgrip MVC), c) an MVC with only the leg contracting (Leg MVC), and d) an MVC with unrestrained leg and pelvis (Unrestrained MVC). Quadriceps torque and activation capacity differences between conditions were compared with repeated measures ANOVA and dependent t-tests, while EMG (from eleven remote muscles) was compared using Friedman's and Wilcoxon. Typical MVC (277.2 ± 49.6 Nm) and Handgrip MVC (261.0 ± 55.4 Nm) were higher than Leg MVC (210.2 ± 48.3 Nm, $p < 0.05$) and Unrestrained MVC (195.2 ± 49.7 Nm, $p < 0.05$) torque. Typical MVC (83.1±15.9%) activation was higher than Leg MVC (68.9 ± 24.3%, $p < 0.05$), and both Typical MVC and Handgrip MVC (81.8 ± 17.4%) were higher than Unrestrained MVC (64.9 ± 16.2%, $p < 0.05$). Only flexor carpi radialis, biceps brachii, triceps brachii and external oblique muscles showed EMG differences, with Leg MVC consistently lower than Typical MVC or Handgrip MVC. Stabilisation of the involved segments is the prime concern subsequently allowing fuller activation of the muscle, reinforcing the need for close attention to stabilisation during dynamometry-based knee joint functional assessment.

INTRODUCTION

Isometric torque produced by maximal voluntary contraction (MVC) of the quadriceps is routinely used to assess knee joint function in various populations, such as clinical (e.g. Hart et al, 1984; Souza et al, 2009) or older (e.g. Reeves et al, 2004; Thompson et al, 2013), as well as to assess the impact of various interventions (e.g Labrunée et al, 2012; Stock and Thompson, 2014). The result of this assessment depends on the muscle size of the subject, as well as their ability to voluntarily activate the knee extensor muscles tested (Kent-Braun and Le Blanc, 1996), with the latter factor being linked to the subject's stabilisation on the dynamometer seat (Hart et al, 1984; Magnusson et al, 1993).

Stabilisation and muscle activation are strongly interlinked, as a more stable segment will allow for higher muscle activation and, consequently, muscle force and torque generation. During quadriceps muscle strength assessment, stabilising the subject and, in particular, their pelvis on the dynamometer seat, allows for greater fixation of the rectus femoris origin (Hart et al, 1984). This facilitates activation and hence greater force production by the rectus femoris muscle, contributing to increased quadriceps torque, as the rectus femoris accounts for ~17% of quadriceps torque (McNair et al, 1991). Similarly, if the pelvis is not adequately stabilised, the bicep femoris muscle is likely to become more active to contribute more substantially towards ensuring stabilisation of the pelvis (van Wingerden et al, 2004). In turn, this will reduce the force generated by the quadriceps muscle, through increased reciprocal neural inhibition by the hamstrings contracting to stabilise the pelvis (Hamm and Alexander, 2010), as well as the increased antagonistic torque.

The activation of the tested agonist muscle, however, can potentially be enhanced through a different path. During an isometric quadriceps MVC, it is common for subjects to simultaneously contract a number of other muscles, remote to the tested muscle, to achieve maximum torque (Jacobsen et al, 2012). When hands were used to hold onto the dynamometer and the back was fixed to it, knee extensors torque was higher by 6.4 % compared to when only the back was fixed, which, in turn, was higher by 7.5% than when no stabilisation at all was used (Magnusson et al, 1993). The activation of those remote muscles may augment the tested muscle's activation capacity, and subsequent torque produced, through a phenomenon termed concurrent activation potentiation (CAP) (Ebben, 2006; Ebben et al, 2008). CAP is underpinned by the theory of motor overflow, which suggests that when a motor area is active, other areas are affected by that activation (Hoy et al, 2004). In the primary motor cortex, which controls movements of the face, arms, and legs (Donohue and Sanes, 1994), activation of one area would also result in higher activation of the others. Indeed, this theory has been supported by studies reporting that contraction of remote muscles to the tested quadriceps, e.g. jaw and arms, results in higher knee extensor torque (Ebben et al, 2008). Interestingly, remote voluntary contractions can also augment the agonistic muscle's torque by increasing stabilisation (for example, hands gripping onto the dynamometer, (Magnusson et al, 1993); the Valsalva manoeuvre increasing intra-thoracic pressure through activation of various torso muscles, stabilising the core, (Harman et al, 1998)) or by directly increasing agonistic muscle activation capacity.

Being able to distinguish between stabilisation and activation effects on torque generation is important to help avoiding erroneous conclusions and difficulty in comparisons between studies of muscle function assessment. Therefore, the aim of the present study was to determine the effect of stabilisation and muscle activation capacity on the quadriceps maximum voluntary isometric torque, by manipulating subject stabilisation configurations on a dynamometer seat and inclusion of simultaneous remote voluntary contractions.

METHODS

Following Institutional ethics approval, nine healthy, active males (mean ± SD: age 28.7 ± 6.8 years, stature 1.78 ± 0.08 m, body mass 89.3 ± 13.0 kg) free from any musculoskeletal injuries gave written, informed consent to participate in the study. In order to reduce variability in performance, all subjects were familiarised with the experimental procedures (Button and Behm, 2004) and visited the laboratory on a single occasion for testing.

Each subject's isometric knee extension strength was initially determined by performing two maximum voluntary contractions (MVC). For those MVCs, the subjects were sat in the chair of a custom-made dynamometer with the hip, knee and ankle joint angles at 90°. The lever arm and the bed of the dynamometer was very rigid, while the restraints allowed for better fixation of the pelvis and the subjects' body compared to commercially available dynamometers. Straps were positioned over the pelvis and tested right thigh, to prevent extraneous movement, while the tested right leg was securely strapped, above the lateral malleolus, to a force-transducer (KAP, E/200 Hz, Bienfait B.V. Haarlem, The Netherlands). If the coefficient of variation (calculated as standard deviation / average * 100) between the two MVCs was <5%, the two MVCs were averaged, otherwise a third MVC was performed and the closest two were averaged (average MVC). Handgrip strength was assessed with the use of a dynamometer (Takei Scientific Inst. Co. Ltd, Niigata, Japan) and the same procedure as for the MVC was followed.

Subsequently, subjects performed an MVC under four different conditions, in a randomised, counterbalanced order, which were:

a) an MVC as described above (Typical MVC),

b) an MVC as the Typical MVC but with the addition of exerting maximal handgrip force (Handgrip MVC),

c) an MVC where the subjects were instructed to isolate the contraction to their leg muscles only (Leg MVC) with the rest of the muscles relaxed, and

d) an MVC where there were no restraining straps on the pelvis and tested thigh (Unrestrained MVC).

During all MVCs, subjects were asked to exert as much force as possible against the ankle strap and had their arms crossed over their chest. Adequate rest between trials was provided.

Force signals were corrected for passive tension of the knee extensors and real-time force readings were displayed online and recorded (Matlab, The Mathworks, Natick, MA). The perpendicular distance from the centre of the knee joint to the point where force was applied (at the level of the ankle, at right angles to the longitudinal axis of the lower leg) was measured and multiplied by that force to provide torque, which was used for further analysis.

Two 7 x 12.5-cm self-adhesive carbon rubber electrodes (Versa-Stim, ConMed, New York, USA) were placed on the proximal and distal regions of the quadriceps muscle group. Stimuli of 200-μs pulse width and 10-ms inter-stimulus gap were generated by an electrical stimulator (model DS7, Digitimer stimulator, Welwyn, Garden City, UK). Electrical stimuli application was displayed online along with the force signal.

Doublets were applied at rest and at increments of 50mA, with the voltage set at 300 V. The intensity that resulted in generating one third of the average MVC torque was recorded and used for the experiment. Subsequently, a doublet was applied at the plateau phase of each MVC (superimposed) and 4 seconds after the superimposed twitch and while the subject was relaxed (resting). Muscle activation capacity was quantified from the superimposed and resting twitch torque using the interpolated twitch technique according to the equation ((1 – (superimposed twitch torque / resting twitch torque))*100.

Two surface Ag-AgCl electrodes of 10mm diameter each were placed in a bipolar configuration on flexor carpi radialis, biceps brachii, triceps brachii (long head), deltoid, pectoralis major, sternocleidomastoid, rectus abdominis, external oblique, vastus lateralis, biceps femoris (long head), and latissimus dorsi muscles to obtain EMG signals. The placement area was prepared by shaving and alcohol cleansing and all electrodes were placed perpendicular to the muscle fibres, with a centre-to-centre distance of 20mm and on the right handside, except for the vastus lateralis where the contralateral muscle was used. These muscles were selected as the more likely muscles to contract during the MVCs described above and, thus, provide an indication of muscle activity during contractions as well as adherence to instructions for the Leg MVC.

EMG was collected at a sampling rate of 2000Hz, and filtered with a high- and low-pass filter of 10 and 500Hz, respectively. The signal was subsequently smoothed using root mean square over 30ms (Aqknowledge, Biopac Systems, Santa Barbara, California) and a mean value from a 500ms window was taken during the plateau phase of the MVC and prior to the application of the twitch. As no comparison between subjects or muscles was to be conducted and testing took place in a single session, no EMG normalisation was performed.

Normality of distribution of the data was checked using Shapiro-Wilk test and subsequently confirmed for handgrip strength, torque and muscle activation capacity but not for EMG. Consequently, a repeated measures ANOVA was used to compare torque and muscle activation capacity between all four MVC conditions, followed by dependent t-test for pairwise comparisons when differences were found. In addition, a dependent t-test was used to compare handgrip strength performed on its own and during Handgrip MVC. Friedman's test was used to compare EMG between conditions for all muscles followed by Wilcoxon test where differences were found. Holm-Bonferroni adjustment was used for all pairwise comparisons and the adjusted p values are presented for these comparisons.

Effect sizes (ES) were calculated for significantly different comparisons to provide an indication of the magnitude of the effect, with 0.8, 0.5 and 0.2 representing large, moderate and small effects for parametric tests effects sizes and 0.5, 0.3 and 0.1 representing large, moderate and small effects for non-parametric tests effects sizes (Fritz et al, 2012). For all statistical analysis IBM

SPSS Statistics v 22 was used. Data are presented as means ± SD, unless otherwise stated. Statistical significance level was set at $p < 0.05$.

RESULTS

Average MVC torque from the two initial MVCs was 298.1 ± 56.7 Nm while submaximal stimulation intensity was 372 ± 123.0 mA.

A significant overall difference was found for torque between the four conditions ($p = 0.001$). Subsequent analysis revealed that Typical MVC was significantly higher than Leg MVC ($p = 0.008$, ES = 1.4) and Unrestrained MVC ($p = 0.004$, ES = 1.7), while Handgrip MVC was also significantly higher than Leg MVC ($p = 0.034$, ES = 1.0) and Unrestrained MVC ($p = 0.008$, ES = 1.3) (Figure 7). In addition, handgrip strength was not significantly different ($p = 0.282$) between handgrip performed on its own (44.4 ± 6.4 kg) and Handgrip MVC (41.6 ± 6.1 kg).

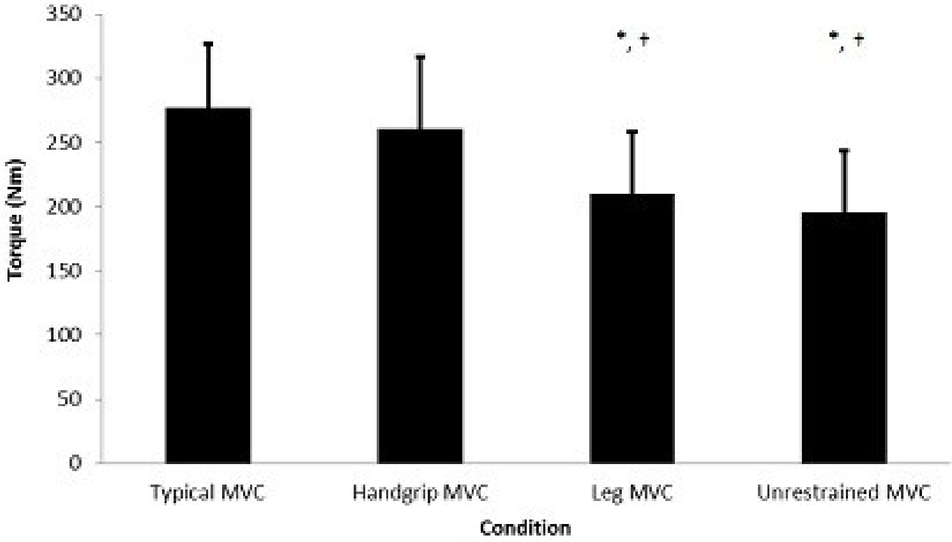

Figure 7. Isometric knee extension torque in all four different conditions. Typical MVC, subjects sat in the dynamometer chair with straps over the pelvis and tested right thigh; Handgrip MVC, as the Typical MVC but with the addition of exerting maximal handgrip force; Leg MVC, subjects were instructed to contract their leg muscles only with the rest of the muscles relaxed; Unrestrained MVC, no restraining straps on the pelvis and tested thigh. Values are means and SD. Significant differences with Typical MVC are indicated by an asterisk, while significant differences with Handgrip MVC are indicated by a dagger symbol.

Muscle activation capacity was significantly different between conditions ($p = 0.001$), with higher activation for Typical MVC compared to Leg MVC ($p = 0.020$, ES = 0.7) and Unrestrained MVC ($p = 0.002$, ES = 1.1), and higher activation for Handgrip MVC compared to Unrestrained MVC ($p = 0.001$, ES = 1.0) (Figure 8). No other differences for activation were found.

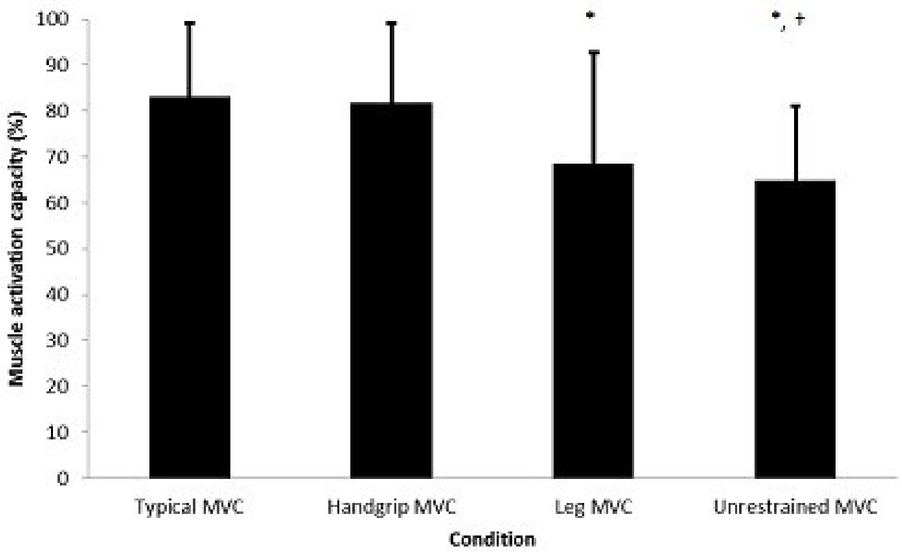

Figure 8. Quadriceps activation capacity in all four different conditions (Typical MVC, subjects sat in the dynamometer chair with straps over the pelvis and tested right thigh; Handgrip MVC, as the Typical MVC but with the addition of exerting maximal handgrip force; Leg MVC, subjects were instructed to contract their leg muscles only with the rest of the muscles relaxed; Unrestrained MVC, no restraining straps on the pelvis and tested thigh). Values are means and SD. Significant differences with Typical MVC are indicated by an asterisk, while significant differences with Handgrip MVC are indicated by a dagger symbol.

EMG differences between conditions were seen for the flexor carpi radialis, biceps brachii, triceps brachii (long head) and external oblique muscles only (Figure 9). For the flexor carpi radialis, Leg MVC was lower than Typical MVC ($p = 0.036$, ES = 0.9), Handgrip MVC ($p = 0.036$, ES = 0.8) and Unrestrained MVC ($p = 0.036$, ES = 0.8). For the biceps brachii, Leg MVC was lower than Handgrip MVC ($p = 0.036$, ES = 0.8) and Unrestrained MVC ($p = 0.036$, ES = 0.8). For the triceps brachii (long head), Leg MVC was lower than Typical MVC ($p = 0.012$, ES = 0.9). Finally, for the external oblique muscle, both Leg MVC ($p = 0.036$, ES = 0.8) and Unrestrained MVC ($p = 0.036$, ES = 0.8) were lower than Handgrip MVC.

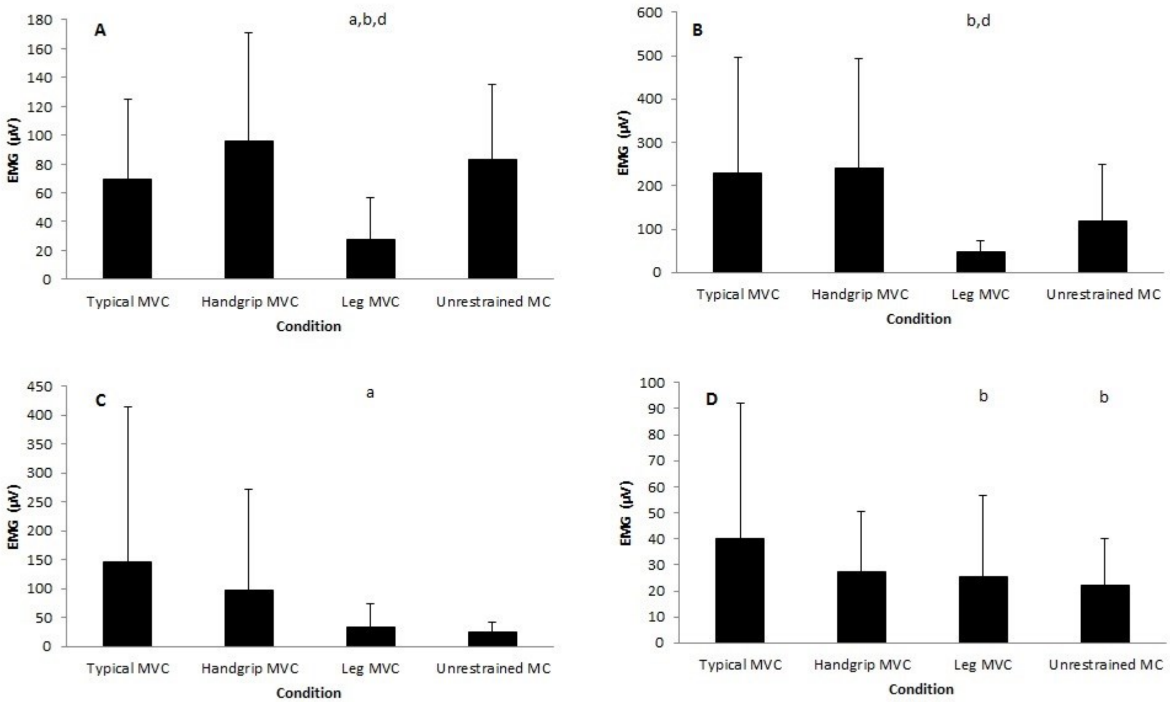

Figure 9. Mean quadriceps EMG in all four different conditions (Typical MVC, subjects sat in the dynamometer chair with straps over the pelvis and tested right thigh; Handgrip MVC, as the Typical MVC but with the addition of exerting maximal handgrip force; Leg MVC, subjects were instructed to contract their leg muscles only with the rest of the muscles relaxed; Unrestrained MVC, no restraining straps on the pelvis and tested thigh) for flexor carpi radialis (Panel A), biceps brachii (Panel B), triceps brachii (long head) (Panel C) and external oblique muscles (Panel D). Values are means and SD. Significant differences are indicated by a, difference with Typical MVC; b, difference with Handgrip MVC; c, difference with Leg MVC; d, difference with Unrestrained MVC.

DISCUSSION

The aim of the study was to examine the effect of subject stabilisation and muscle activation capacity on knee joint torque developed during an isometric MVC, by distinguishing the effect of each component through manipulation of stabilisation configurations and inclusion of remote voluntary contractions. The results suggest that although both stabilisation and activation capacity play an important role in force generation, stabilisation of the involved segments plays the major role which will in turn allow fuller activation of the muscle.

When the handgrip was added to the Typical MVC, no statistically significant change in torque or muscle activation was observed. These results agree with CAP literature showing that when bilateral handgrip was added to knee extension, torque from an isometric contraction (Ebben et al, 2009) or dynamic contraction (Cherry et al, 2010) did not change, suggesting no beneficial effect of handgrip on knee extensor torque. Similarly to the present study, handgrip strength was also not significantly reduced during the knee extension (Cherry et al, 2010; Ebben et al, 2008). These findings contradict expectations of increased activation and subsequent torque due to increased H-reflex activity and motor-evoked potentials induced by the additional handgrip contraction (Dowman and Wolpaw, 1988; Péréon et al, 1995).

One possible reason for this contradiction is the contraction of the handgrip-related muscles not being sufficient to excite further the difficult to activate (possibly due to its higher content of type II muscle fibres; (Johnson et al, 1973)) quadriceps muscle (Behm et al, 2002), as suggested by the very similar activation values during Typical MVC and Handgrip MVC. A second possible reason relates to the action performed with the handgrip. When the arms were used to grab the dynamometer seat, an increase in knee extension torque was seen, attributed to a better-fixed torso (Magnusson et al, 1993). However, the handgrip contraction used in the present study and Ebben et al (2008) and Cherry et al (2010) studies, does not appear to substantially contribute towards stabilising the torso. Therefore, although excitatory responses may take place during the grip, these do not assist in further stabilising the pelvis during knee extension. This notion is supported by findings that gripping the dynamometer seat or the pelvic strap during knee extension had no effect on quadriceps torque (Kramer, 1990), most likely due to the fact that both actions offered the same stabilising effect to the torso.

When the subjects were requested to focus on contracting the leg muscles only (Leg MVC) while still restrained by the dynamometer belt, activation was reduced by 17.5%, while torque was reduced by 24.2% when compared to the Typical MVC values. EMG data suggests that subjects were able to ‘engage less’ the flexor carpi radialis, biceps brachii, triceps brachii (long head) and external oblique muscles, as the EMG values were lower during the Leg MVC. In a study examining the EMG activity of biceps femoris (long head), external oblique and rectus abdominis muscles during an isotonic exercise, however, those muscles were shown to have lower activation during the isotonic exercise (Jacobsen et al, 2012). If this was the case in the present study, it is then unlikely that the reduction in quadriceps activation values was due to the reduced EMG activity of those muscles. Interestingly, the rest of the EMG data showed no difference between any of the conditions. This could mean that other muscles were not required for the contraction, and hence, they remained ‘quiet’ throughout all conditions, or they were crucial to the contraction and therefore they were activated to achieve the task required, regardless of the instruction. Whichever the reason, the lack of difference in EMG activity between conditions for the rest of the muscles studied, precludes them as contributors to the muscle activation capacity changes.

When the pelvis and tested right thigh restraints were removed (Unrestrained MVC), the reduction in activation (22.0%) and torque (29.6%) compared to typical MVC, was higher than the respective reduction in activation and torque seen in Leg MVC condition, although not statistically significantly so. Given that the subjects’ EMG in the measured muscles during Unrestrained MVC was equal or higher than the corresponding EMG values during Leg MVC, it is reasonable to assume that CAP did not augment knee extensor torque, as otherwise activation and torque would be higher in the Unrestrained condition. Collectively, these findings suggest that pelvis and tested thigh stabilisation is the major factor determining the knee extensor torque produced during an isometric knee extension, and optimal stabilisation subsequently facilitates muscle activation enabling maximum possible force generation by the tested muscles.

The subjects in the present study were familiar with maximal isometric contraction, as per Typical MVC. However, the Leg MVC condition inevitably contained two potentially conflicting instructions (‘push as hard as possible against the ankle strap’ and ‘use only your leg muscles, relax the other ones’). This could have presented a limitation to the force generation during this condition as the opposing instruction requirement could impact negatively on maximum force generation (Marchant, 2010). In addition, during the Unrestrained MVC, there was a tendency for subjects to lift off the dynamometer seat. However, they all maintained a position similar to the Typical MVC. It is likely that this position was maintained by voluntary activation reduction to prevent further lifting off the chair, which supports further the concept of the need for stabilisation first to enable maximum voluntary activation of the muscles.

In conclusion, the present findings suggest that although stabilisation and activation are interlinked, stabilisation of the pelvis during an isometric knee extension is a priority in order to allow maximum voluntary activation of the quadriceps muscle. These results further reinforce the need for close attention to stabilisation during dynamometry-based knee joint functional assessment.

CHAPTER 7

DISCUSSION

The aim of the present investigation was to examine the effect of the mechanical behaviour of the quadriceps muscle on muscle activation capacity and the methodological implications this has. The results show that this aspect not only can affect the assessment results through change of muscle-tendon stiffness and stabilisation (impacting on activation capacity) but it can also contribute to implementing the assessment with decreased discomfort for the subject or patient.

The stiffness of muscle-tendon unit regulates how faithfully force is transmitted, and as such, it can have a great impact on activation capacity results based on calculations of the twitch force magnitude (Oda et al, 2007). Loring and Hershenson (1992) demonstrated that a more compliant link resulted in a lower adductor pollicis twitch force magnitude compared to non-compliant link, suggesting that a more compliant muscle-tendon unit, will result in a smaller twitch force. The results of Chapter 3 support that notion, with lower ITT values obtained when the knee joint angle was at 90 degrees (stiffer muscle-tendon unit) compared to 30 degrees (slacker muscle-tendon unit), due to consideration of the resting twitch, while no differences between knee joint angles were found when using the CAR method, due to consideration of the superimposed twitch only.

The consideration of the resting twitch with the ITT method poses another possible limitation, in terms of the muscle fibres activated during the superimposed and resting twitches. The superimposed twitch is delivered to a contracted muscle, and likely, therefore, to a larger number of muscle fibres, as they will have shortened during the isometric contraction and more muscle fibres will have been present under the electrode. During rest, however, the muscle fibres will have returned to their resting status, thus changing the amount of muscle fibres under the electrode (Arampatzis et al, 2007). As suggested by Chapter 4, activating the same portion is an important factor in percutaneous muscle activation, and as a result, CAR not taking into account the resting twitch avoids both the above issue as well as increased tendon slackness at rest (Herbert et al, 2002; Ohta et al, 2009).

These results must be considered when muscle activation capacity is assessed. Comparing different groups of participants, e.g. young and older (Karamanidis and Arampatzis, 2006; Onambele et al, 2006) or healthy and unhealthy (e.g. Maganaris et al, 2006) must be done with caution, and the methods used for assessing activation capacity should reflect the implications of altered muscle-tendon stiffness, to obtain a true reflection of the changes that took place. This suggestion is perhaps more pertinent, when interventions are taking place and pre-post measurements are needed. For example, Kubo et al (2002) implemented an 8-week resistance only and combined resistance and flexibility training programme in young adults (21 years) and reported increases in triceps surae stiffness of 18.8% and 15.3%, respectively. Similarly, patella tendon stiffness of older individuals (74.3 years) increased following a strength training programme by 65% - 168%

(over a range of 10% intervals of maximal tendon force) (Reeves et al, 2003). Even in shorter interventions, fatiguing exercises have been shown to alter the architectural properties of the muscle (Maganaris et al, 2002; Thomas et al, 2015), and as force generation is the result of muscle fibre and tendon interaction (Kawakami and Lieber, 2000; Oda et al, 2007), potentially altering the outcome of muscle assessment capacity.

The impact that altered muscle-tendon unit stiffness has on muscle activation capacity assessment was further highlighted when different stimulation intensities were applied in Chapter 4. Valid activation capacity results were only possible when the muscle-tendon unit was sufficiently stretched by the higher stimulation intensity, while lower stimulation intensities, which were unable to do so, yielded erroneous results. That stimulation intensity (approximately 50%) resulted in a constant superimposed twitch to resting twitch ratio.

Sufficient stretching of the muscle-tendon unit with increasing stimulation intensity may be due to the increased intensity itself, resulting in the muscle portion directly under the electrode to become more activated, or it could also be due to the increased electrical field. This increase with the higher stimulation intensities, is likely to activate the deeper parts of the muscle (Binder-Macleod et al, 1995), resulting in 'more muscle' contracting during the increasing stimulation intensity. This increased activation during the superimposed twitch may not be reflected in the resting twitch, as at rest the tendon slackness will be unlikely to be stretched at the same level as during contraction (Herbert et al, 2002; Ohta et al, 2009). This also appears to agree with suggestions that contraction intensities of >75% MVC are needed to achieve sufficiently accurate predictions (Behm et al, 1996).

Stimulation intensity is a parameter that has been suggested as an important factor in electrical stimulation programmes that are used with patients (Doucet and Griffin, 2008), aiming to increase strength (Valli et al, 2002). The need for increased intensity to achieve valid results, however, is associated with increased feelings of discomfort in subjects (Delitto et al, 1992; Chae et al, Valli et al, 2002), and submaximal stimulation intensities have been used to account for that (Valli et al, 2002).

This discomfort has been shown to lead to reduction of effort prior to the application of the stimulus (Button and Behm, 2008), and it has been reported that pain results in decreased force generation (Pfingsten et al, 2001). The findings of this study, therefore, have two direct applications in the use of electrical stimulation. Firstly, the identification of a threshold which can be used as a practical guide by researchers and practitioners to apply submaximal stimulation intensity with confidence that the muscle activation capacity outcome is valid. Secondly, the associated decrease in discomfort at that threshold felt by the subjects, which can address some of the methodological concerns induced by supramaximal stimulation intensity as well as ethical concerns in terms of the subject discomfort and drop-out (e.g. Behm et al, 2001; Miller et al, 2003; Han et al, 2006).

The force-length curve (Gordon et al, 1966) dictates the force-generating capacity of a muscle at different lengths. With a muscle operating on the ascending limb of the curve, force will increase with elongation of the muscle. Conversely, if a muscle operates on the descending limb of the curve, then force productions will decrease with further elongation. Finally, force production is relatively stable at the plateau region (Arnold and Delp, 2011). Based on Gordon et al (1966) theory, several musculoskeletal models were developed to describe how the human muscles would operate (e.g. Herzog and te Keurs, 1998; Herzog et al, 1990). These models, however, contradict experimental findings (Maffiuletti and Lepers, 2003; Rochette et al, 2003). Specifically, the biceps femoris operates on the ascending limb of the force-length curve and, thus, when in the supine positions it would be elongated, increasing the force output of the quadriceps muscle. The theoretical outcome not being verified by experimental findings could be attributed to two reasons, differences in activation capacity (Maffiuletti and Lepers, 2003) or differences in antagonistic co-activation, affecting the ‘net’ knee extension outcome (Maganaris et al, 1998). The present results in Chapter 5 show that the discrepancy between the musculoskeletal models and experimental studies cannot be attributed to antagonistic co-activation, as antagonistic co-activation was higher in the seated position compared to the supine. The decreased antagonist co-activation, combined with the decreased agonist activation at that position can be explained by the common drive hypothesis (Basmajian and De Luca, 1981), which suggests that the quadriceps and hamstrings activation will alter concurrently, due to their common central drive, possibly in an attempt to stabilise the knee joint (Solomonow et al, 1989).

The results of the present study contradict findings by Kubo et al (2004), reporting higher antagonistic co-activation at the shorter hamstring muscle length. A reason for this discrepancy may be the different joint manipulated in the two studies. In a study examining the effect of knee and hip joint changes on the biceps femoris (long head) fascicle length and the force generated (Chleboun et al, 2001), it was reported that biceps femoris fascicle length was more sensitive to changes in the hip joint than it was to changes in the knee joint, possibly due to greater moment arm at this joint (Visser et al, 1990).

The increased agonist activation at the seated position as well as the concurrently reduced agonist activation and antagonist co-activation, point towards inability to completely fix the pelvis during contraction. In the present study, we employed additional blocks to increase stabilisation, in an attempt to avoid extraneous movement. However, unless the pelvis is completely stabilised, then stabilisation equal to the one achieved in the seated position, is unlikely. Consequently, this will impact negatively on the subjects' ability to exert maximal force (Hart et al, 1984; Magnusson et al, 1993), thus reducing the torque output.

This interaction of stabilisation and ability to maximally activate the muscles, was examined in Chapter 6, by manipulating fixation (by including / removing the stabilising straps) as well as purposely increasing (through remote voluntary contractions; Ebben, 2006) and reducing (through focusing on activating the contracting leg only) muscle fibre recruitment. Predictably, our results showed a decrease in torque and activation capacity when the straps were removed and when the subjects were instructed to focus on contracting the tested leg only. When the two conditions were compared, the EMG values of the tested muscles were equal or higher during the unrestrained condition compared to the conditions where only the leg was contracted. Despite this, both torque and activation were lower in the unrestrained condition (although not statistically so). As the above findings preclude concurrent activation potentiation (Ebben, 2006), the conclusion that can be drawn is that stabilisation of muscles is the major concern during muscular contraction, which will in turn facilitate fuller activation. These findings are important in standardising muscle function assessment with the use of a dynamometer, in order to obtain more accurate assessment results as well as compare between studies.

The present studies focused on manipulation of experimental set up aimed at impacting on the muscle mechanical behaviour. By doing so, however, certain physiological parameters also changed and could potentially have an effect on the results seen. Specifically, longer muscle lengths (as in Chapter 3 and Chapter 6), would have increased calcium availability (Balnave and Allen, 1996), subsequently increasing the sensitivity of submaximally recruited muscle fibres. In addition, muscle length changes will have also affected the muscle spindle activity. With longer lengths, muscle spindles would have increased neural activation, through an increased afferent Ia discharge rate (Becker and Awiszus, 2001). This, in turn, would result in higher excitation (Becker and Awiszus, 2001; Roatta et al, 2002) and, therefore, higher activation.

The combination of the findings from the four experimental chapters demonstrates the effect muscle mechanics can have on muscle activation capacity assessment. Not accounting for the ability of the muscle to faithfully transmit force, either through better fixation or through altered muscle-tendon stiffness, can impact on the results. Similarly, reduction of discomfort through a stimulation intensity sufficient to achieve faithful transmission of force as well as appropriate testing position enables achievement of results closer to true maximum. Subsequently, consideration must be given to these aspects to ensure a more accurate assessment. The following recommendations take into account these findings and offer a guide for muscle activation assessment capacity:

1. Conduct testing in the seated position.
2. Ensure maximum stabilisation to reduce compliance of muscle-tendond unit.
3. Use the stimulation intensity that generates at least 1/3 of MVC.
4. Instruct the subject to contract maximally (allowing contraction of remote muscles).
5. If change of muscle tendon compliance is suspected / expected, use CAR to calculate muscle activation capacity whereas if no change of muscle tendon compliance is suspected / expected, use ITT to calculate muscle activation capacity.

The application of current through percutaneous electrical stimulation presents some limitations, most notably the possibility of co-contraction of agonist and antagonist muscle due to current spread (Alon et al, 1994; Taylor et al, 2009) and antagonist muscles through activation of cutaneous receptors (Belanger and McComas, 1981; Poumarat et al, 1991) or discomfort (Paillard et al, 2005). Although extreme care was taken with electrode placement (Shield and Zhou, 2004) to reduce this effect, the present studies were ultimately subject to the same problem. The current results could help alleviate some of these concerns in terms of antagonistic co-contraction and discomfort affecting the outcome. Antagonistic co-contraction can be reduced with lower stimulation intensity (Shield and Zhou, 2004), thus providing a more accurate picture of a muscle's stimulation capacity. Further, as older individuals feel pain at a lower stimulation intensity than younger (Valli et al, 2002), the use of submaximal stimulation intensity may assist this group's assessment as well as provide a more accurate picture of the activation deficit between young and older.

Inter-subject variability could potentially have had an effect on the results. For example, in Study 2, the individuals' levels of activation plateauing varied, with some achieving a plateau as low as 30% stimulation intensity. As explained earlier, a stiffer muscle-tendon unit will be sufficiently stretched earlier (and, thus, need lower stimulation intensity to achieve comparable results to supramaximal intensity). It is possible, therefore, that some subjects with a stiffer muscle-tendon unit will require lower stimulation intensities. The practical criterion of using a stimulation intensity that will achieve force generation equal to one third of MVC, provides a generic solution to this problem.

Inter-subject variability could also impact in another way. The results of all four studies are based on sample sizes ~10 subjects. Recruitment of subjects was based on availability; therefore the participants used in each study are different individuals. Although similar sample sizes have been used in previous studies (e.g. Behm et al, 2001; Ebben et al, 2008; Maffiuletti and Lepers, 2003), it is possible that the smaller sample size, along with the increased standard deviation expected in such studies (Allen et al, 1995; Babault et al, 2003), may have impacted negatively on the studies' power. Therefore, the conclusions must be interpreted with some caution.

It has also been reported that subject variability in the operating range of the rectus femoris varies in healthy but untrained individuals (Winter and Challis, 2010). From 28 subjects used in that study, 14 operated on the ascending limb of the force-length curve, 7 over the plateau region and 9 subjects on the descending limb (Winter and Challis, 2010). In Study 3, tetanic stimulation being higher in the supine position, compared to seated, suggested that all our subjects were operating on the ascending limb of the force-length curve. However, such variability poses limitations on the generalisability of the findings and should be considered in future studies examining mechanical effects through hip joint manipulation.

Finally, the effect of the gastrocnemius muscle was not considered in the present studies. The gastrocnemius is a bi-articular muscle crossing over the ankle and knee joints, and is affected by the knee joint angle (e.g. Landin et al, 2015; Baumbach et al, 2014). As the ankle joint angle affects the torque and activation of the gastrocnemius (Maganaris et al, 1998), future studies should examine the effect of the gastrocnemius antagonistic co-activation in different positions during isometric knee extension torque, to allow a better understanding of the mechanical and neural effects impacting on knee extension torque during assessment.

In addition to examining the effect of gastrocnemius antagonistic co-activation on isometric knee extension torque, the findings of the present Thesis generate some more questions for further research in the area. The activation level of the quadriceps muscle during an isometric knee extension has been examined at various ranges of knee joint angles (e.g. 30° - 90° with 5° increments, Becker and Awiszus, 2001; 10° - 110° with 20° increments, Newman et al, 2003; 40° - 110° with 10° increments, Kubo et al, 2004) reporting somewhat contradicting results. Newman et al (2003) reported no effect of knee joint angle on muscle activation capacity, while both Becker and Awiszus (2001) and Kubo et al (2004) studies, reported an increase an activation at longer muscle-tendon unit lengths, albeit with different patterns of muscle activation capacity increase from shorter to longer muscle-tendon unit lengths. The present results have demonstrated a change in muscle activation capacity of the quadriceps muscle with alterations in the knee and hip joint angles and the effect of stabilisation on activation and force generation. It would be of interest to examine the effect of stabilisation on quadriceps muscle activation capacity throughout the knee joint angle range as a possible reason for the discrepancy seen in the above studies. Such assessment of the quadriceps muscle would allow for a more accurate development of torque curves, and provide information on what affects muscle force at each angle (Ikeda et al, 2002).

Further, the quadriceps muscle has been examined as a group, with little consideration to the individual muscles the quadriceps consists of. The present results, however, have demonstrated that altering the behaviour of the rectus femoris can affect the quadriceps muscle torque output. Considering that the vastus lateralis appears to be more affected by fibre atrophy in older individuals compared to the vastus medialis (Boccia et al, 2015), as well as that the four muscles making up the group generate force differently based on the knee joint angle (Herzog et al, 1990), further research on each muscle would provide relevant information on effective intervention strategies (Leroux et al, 1997). The function of the rectus femoris, vastus lateralis and vastus medialis has been investigated via electrical muscle stimulation (Leroux et al, 1991), hence providing guidance on stimulating these muscles individually.

In summary, the present studies have shown that quadriceps muscle activation capacity assessment can be affected by the mechanical behaviour of the muscle group. These findings can help to ensure more accurate assessment of muscle activation capacity through use of appropriate stimulus and intensity, consideration of the likely changes following an intervention and the factors that can contribute or not to that assessment.

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APPENDIX 1

APPENDIX 2